Blinded Abstract Body

Limit 5 pages not including the Appendices (single-spaced, Times New Roman, 12 pt. font, 1 inch margins)

A gut pathobiont triggers neuroinflammation and neurocognitive impairment via breaching the gut-brain axis in a preclinical model of Alzheimer's disease: from microbial pathogenesis to neuropathogenesis

Purpose

Description of the objective or focus of the research.

The objectives of this study were to test the hypothesis that antibiotic-induced enteric *Klebsiella pneumoniae* (Kpn) pathogenesis triggers or aggravates neuroinflammation and neurocognitive impairment associated with AD neuropathogenesis through the impairment of the gut-blood-brain axis. Additionally, the study aimed to identify co-occurring bacterial and fungal taxa in the intestinal, systemic, and neuronal niches that underlie and contribute to the colonization and gut-brain translocation of Kpn during the progression of AD.

Background and Context

Description of prior research and its intellectual context.

Kpn is notorious for causing nosocomial infections and is commonly found in elderly patients in hospitals with Alzheimer's disease (AD)[1]. This bacterium can cause bloodstream infection, which may worsen AD pathophysiology, but there are no studies demonstrating the mechanistic role of this pathogen in AD.

Method

Description of the research (include a description of the study design, research location, participants, intervention, and methods of collecting and analyzing data if applicable).

We infected APP/PS mice intestinally with Kpn with (Kpn+Ab) and without antibiotics (Kpn) for a week. We subsequently collected fecal and serum samples at 1 and 3 weeks and Neurocognitive function and motor coordination were assessed, followed by the collection of intestinal and brain tissues at 5 weeks. Bacterial and fungal communities were evaluated through 16S rRNA amplicon sequencing, while metabolites in the gut, serum, and brain were quantified using NMR spectroscopy-based untargeted metabolomic analysis.

Findings or Results

Description of the main findings with specific details.

Remarkably, the presence of antibiotics led to a sharp rise in gut Kpn levels, inversely correlated with microbial diversity. Conversely, Kpn was scarcely detected in the non-antibiotic Kpn group, highlighting the role of antibiotic-induced gut dysbiosis in triggering a unique 'pathobiome' signature. Notably, we observed a substantial increase in *Candida* levels within both the gut and the serum of the Kpn+Ab group. This escalation serves as indicative evidence of *Candida* translocating from the gut into the bloodstream, implying the potential for bloodborne infection to trigger cerebritis and subsequently lead to memory deficits. Five weeks post-infection, Kpn showed a greater affinity for colonizing the small intestine compared to the large colon. Notably, the Kpn+Ab group exhibited the presence of Kpn not only in the bloodstream but also in the brain, indicating intestinal-to-brain Kpn translocation via circulation. In cases where Kpn was found in

the brain, substantial neuroinflammation resulting from bacterial infection was evident. Meanwhile, the level of short chain fatty acids, particularly acetate, was found to be reduced in the gut of the Kpn+Ab group, a consequence of dysbiosis. Additionally, in the brains of the Kpn-treated groups, there was an observed tendency for aspartate and N-acetyl aspartate to increase, while taurine and lactate, both of which play a role in modulating brain function, exhibited a tendency to decrease. Subsequent neurocognitive and behavioral assessments revealed impaired memory function and motor coordination in Kpn-infected mice, particularly those in the Kpn+Ab group.

Conclusions and Implications

Description of conclusions/recommendations based on findings, potential impact/importance to your field, and limitations.

These results corroborate the emerging notion of the implicating role of the gut dysbiosis and consequent gut-brain axis impairment in Alzheimer's neuropathology. Our findings suggest that Alzheimer's patients who are hospitalized and treated with antibiotics are at a higher risk of contracting multi-drug resistant Kpn. Further, these data also hint that gut pathobiome may increase the host's predisposition to AD by breaching the gut-brain axis thereby triggering neuroinflammation and impairing neurocognitive function.

Appendix A - References

Not included in page count.

1. Young, T. M., Bray, A. S., Nagpal, R. K., Caudell, D. L., Yadav, H., & Zafar, M. A. (2020). Animal model to study Klebsiella pneumoniae gastrointestinal colonization and host-to-host transmission. Infection and immunity, 88(11), 10-1128.

Appendix B - Tables and Figures

Not included in page count.

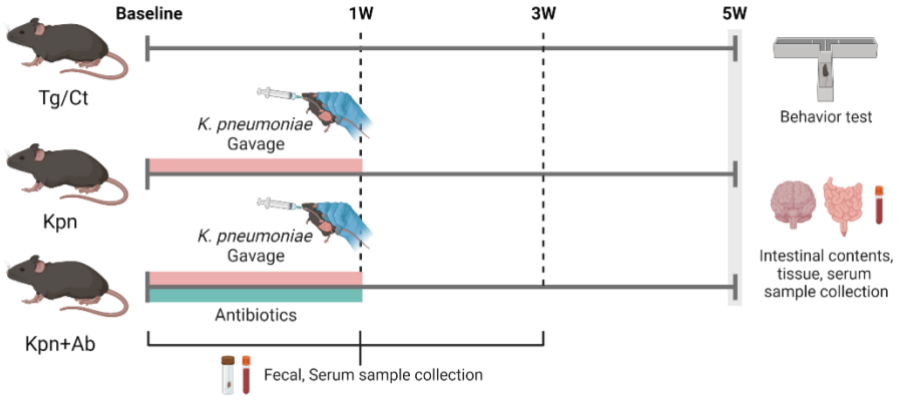

Figure 1. Study Design

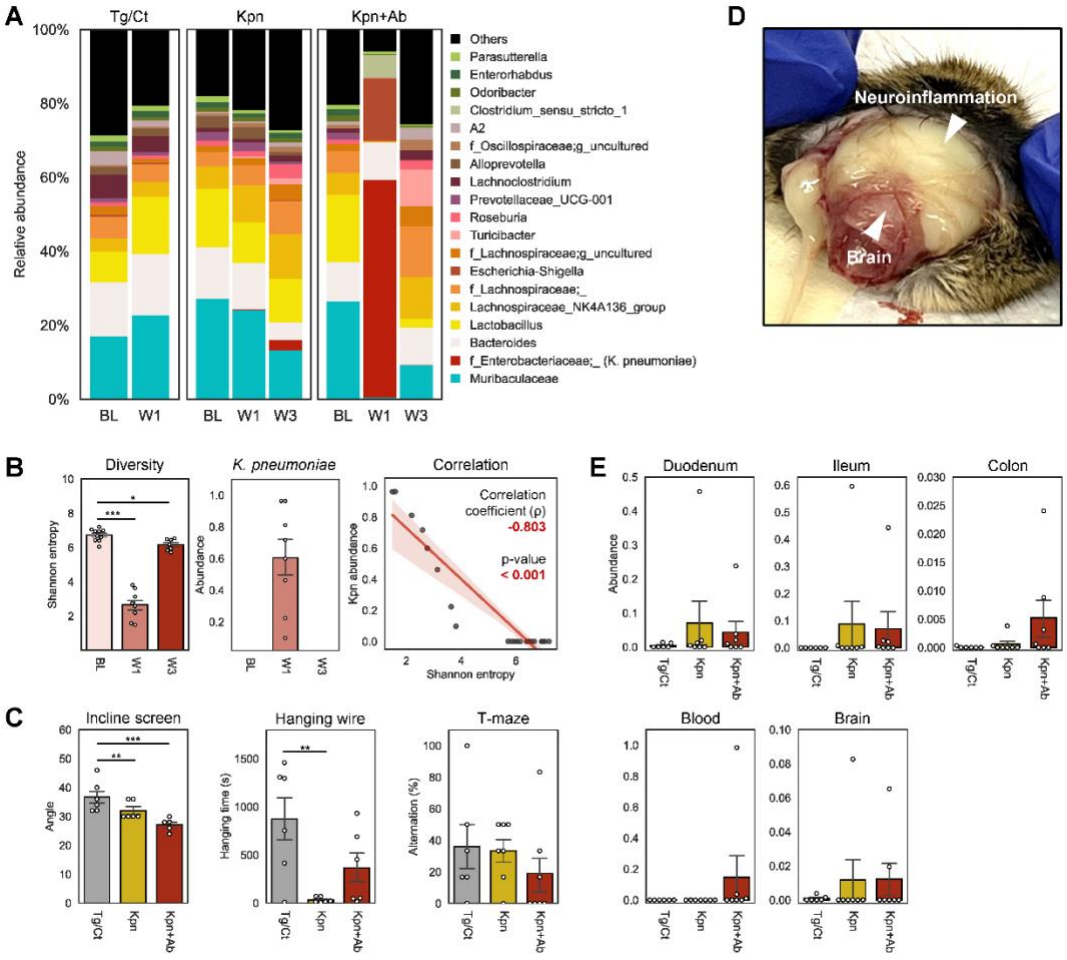

Figure 2 Effects of *Klebsiella pneumoniae* (*K. pn*) colonization. **A.** Changes in microbial composition before and after *K. pn* colonization, **B.** Correlation between microbial diversity (Shannon entropy) and abundance of Kpn of Kpn+Ab group **C.** Assessment of motor coordination (incline screen and hanging wire test) and memory function (T-maze) test. **D.**

Neuroinflammation after *K. pn* translocation from gut to brain, E. Abundance of *K. pn* in the three parts of small intestine, colon, blood, and brain.