Peer Review File

Article information: https://dx.doi.org/10.21037/jtd-23-493

Reviewer A

1. In addition to only using CCK-8 kit to test the cell viability, validating the Caspase-3 activity or performing an MTS assay with the untreated group is necessary to strengthen the arguments.
RE: Dear reviewer, we have supplied western blot to access the activity of caspase-3 in order to strengthen the arguments.

2. In Fig.3, the authors should quantify the intensity of the F-actins staining before and after infection by providing a bar graph with individual data points for each group.
RE: Dear reviewer, we have added a bar graph for each group.

3. Please provide a high-resolution figure for Fig 4; the characters on 4a, 4c, and 4d are not visible.
RE: Dear reviewer, we have replaced Fig 4 with a high-resolution figure.

Minor:

1. Fig.1a is plotted as a bar graph with individual data points, but 1b and 1c use a simple bar graph. Please unify the format.
RE: Dear reviewer, we have revised the figure.

Reviewer B

The paper titled “K. pneumoniae and M. smegmatis infect epithelial cells via different strategies” is interesting. The results emphasized the immunoprotection and immunomodulation of lung epithelial cells against exogenous pathogenic microorganisms, indicating that different pathogens damaged the host through different strategies and induced varying innate immune responses. At the same time, they provided important clues and key immune factors for dealing with complicated pulmonary infections. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) How do lung epithelial cells activate the genes required for intracellular survival and present their pathogenicity? How do these two bacteria play a role in this process? It is recommended to add relevant content.

RE: Dear reviewer, thank you for your advice. Due to the limitations of laboratory conditions, we only detected the differentially expressed genes after infection, and the potential mechanisms still remains unclear. We will explore the mechanism of them by *in vivo* experiments in next study and we added this problem in our manuscript.

2) Figures 4-5 are not clear enough. It is recommended to provide clearer figures again.

RE: Dear reviewer, we have replaced Fig 4 and 5 with high-resolution figures.

3) The bioinformatics analysis of this study is too simple, and it is suggested to add relevant comparative analysis.

RE: Dear reviewer, we have supplied bioinformatics analysis such as relevant comparative analysis.

4) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Detection of Klebsiella pneumoniae cfDNA in pleural fluid and its clinical value, PMID:32954758". It is recommended to quote the article.

RE: Dear reviewer, thank you for your suggestion, and we have quoted the article.

5) Pulmonary oxidative stress and neutrophil activation are important factors in the development of respiratory failure after acute lung injury and sepsis. How do these two bacteria play a role in these processes? It is recommended to add relevant content.

RE: Dear reviewer, thank you for your suggestion and we supplied the content about the roles of *K. pneumoniae* and *M. smegmatis* in lung injury and sepsis.

6) Why are these two bacteria selected for comparative analysis? What are the biggest similarities and differences? It is suggested to add relevant contents.

RE: Dear reviewer, *K. pneumoniae* is the Gram-negative respiratory pathogen and *M. smegmatis* is the Gram-positive respiratory pathogen. Both *K. pneumoniae* and *M. smegmatis* can effectively "escape" the effect of antibacterial drugs. We would like to understand mechanism in terms of the complex interaction between different kinds of bacterial pathogens and their host cells. As a result, we chose them for comparative analysis.

7) What is the author's next research plan? What is the inspiration for dealing with complicated pulmonary infection?

RE: Dear reviewer, to verify our findings, more research with i*n vivo* models that more closely resemble pulmonary tissues is required. Research based on co-infection with a 2–3 pathogens model will be performed in the future.