

Peer Review File

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Reviewer A

General comments: I think this paper is well written and worth publishing. However, I think there are few points to be improved.

Major suggestions:

1. On introduction, authors mention that extra-genitourinary *Mycoplasma hominis* infections usually occur in immunocompromised patients. However, the reported cases were not immunocompromised, and the reason for this discrepancy was not explained. The authors must discuss on this point if they mention about it in the introduction.

Reply 1: In previous literatures, these infections usually occur in immunocompromised patients. However, extra-genitourinary infections of *M. hominis* are rare and the number of reported cases is limited. Patients with extra-genitourinary infections of *M. hominis* have significant heterogeneity and this disease lacks sufficient study. In our study, the seven reported cases of *M. hominis* mediastinitis were not immunocompromised, expanding our understanding of this disease.

Changes in the text: Page 3, Line 84-88; Page 4, Line 110-112.

2. Although some previous reports mention about postoperative *Mycoplasma hominis* mediastinitis, the authors did not mention about it in the introduction. Since it is an important background that causes *Mycoplasma hominis* mediastinitis, could the authors kindly mention about it in the introduction as well?

Reply 2: In previous literatures, a large portion of extra-genitourinary infections are postoperative infection.

Changes in the text: Page 3, Line 82-83.

3. p8 line 262: I am not sure whether conduction of mNGS was delayed in patient D. On what basis did the authors determine that there was a "significant delay"? Are there any arguments on adverse outcomes and concurrent infections?

Reply 3: To some extent, the high cost of mNGS has limited its clinical application in China. Not all the patients are financially capable of payment of mNGS testing fees. For patient C and D, at the beginning of the worsening of their conditions, clinicians had already recommended mNGS to their family. Their family hesitated for financial reasons. By the time they decided to undergo mNGS testing, the infection had worsened, and the patients subsequently died.

For patient D, mNGS was recommended POD12. Although the patient died of multiple organ failure, partly due to surgical trauma, infection is still the most important reason for his condition worsening. *A. baumannii* seems to be less virulent than *M. hominis* and might not be the main cause of death.

Changes in the text: Page 9-10, Line 312-321.

Minor suggestions:

p6 line 186: I think it is not possible for authors to say "no difference" from this result. I suppose "no significant difference" is the accurate term.

Reply: We have modified our text as advised.

Changes in the text: Page 7, Line 234.

Reviewer B

- 1) First, I suggest the authors to indicate the research design of this study in the title, i.e., a case serial and literature review.

Reply 1): We have modified our text as advised.

Changes in the text: Page 1, Line 3-4.

- 2) Second, the abstract needs some revisions. The background did not explain why mNGS could address the clinical concern. The methods need to describe the inclusion of the seven cases, their clinical presentations, treatment, and prognosis. The results need to briefly summarize the findings from the literature review. The current conclusion needs to be made with cautions because the authors only reported successful cases detected by using mNGS.

Reply 2): We have modified our text as advised.

Changes in the text: Background: Page 1, Line 29-32. Methods: Page 2, Line 38-41. Clinical presentations, treatment, and prognosis were added in results: Page 2, Line 50-54. Results: Page 2, Line 55-57. Conclusion: Page 2, Line 58.

- 3) Third, in the introduction, the authors need to analyze why mNGS is seldom used in diagnosing *M. hominis* mediastinitis and the strengths of mNGS for *M. hominis* mediastinitis.

Reply 3): We have modified our text as advised.

Changes in the text: Page 3, Line 104-106.

- 4) Fourth, in the methodology of the main text, please clearly indicate the clinical research design of this study such as a case serial and consider to report it according to the CARE guideline. For the literature review part, please specify the literature search and describe how the retrieved studies were analyzed. In statistics, please directly describe all the analyses are descriptive and there is no need to describe $P < 0.05$ since there were no comparisons.

Reply 4): We have modified our text as advised. *M. hominis* detection results by mNGS were compared between serum and body fluid. The SMRNG, SMRN, and coverage rate of *M. hominis* detection by mNGS were significantly higher in body fluid (abscess or mediastinal fluid) than in serum ($P < 0.05$), as shown in *Table 3*. There was no significant difference in relative abundance of genera or species of *M. hominis* detection between serum and body fluid (Page 7, Line 230-235).

Changes in the text: Page 4, Line 117. Page 4, Line 126-128.

Reviewer C

1. Table 4

Please explain “-” in table footnote.

Patient no.: age (years), gender	As:
1: -, M	Co: lun
2: -, M	CF

Response: We have explained “-” in table footnote.

2. Please confirm if citations are needed in this sentence, as you mentioned “previous studies”.

*For bacteria, viruses, and parasites, the criteria for a positive mNGS result were as follows: mNGS identified a bacterium, virus, or parasite of species level of which the coverage rate scored 10-fold greater than that of any other bacteria, viruses, or parasites according to *previous studies*.*

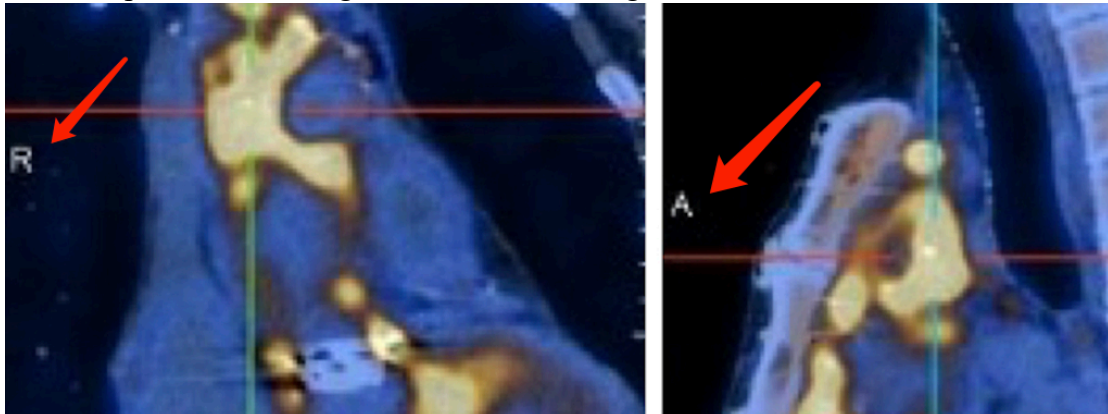
Response: We have added the citation (Main manuscript Page 6, Line 175).

3. The citation of Ref. 25 in the main text was missing. Please indicate where you would like to cite Ref. 25 in the main text. Please note that it should be cited between Ref. 24 and Ref. 26.

Response: We have modified the text as advised (Page 9, Line 275).

4. Figure 1

Please explain the meaning of “A”. “R” in the legend.



Response: We have modified the text as advised (Page 16, Line 502-503).