PEER-REVIEW REPORT

Name of journal: World Journal of Clinical Cases

Manuscript NO: 63840

Title: Clinical Metagenomic Sequencing for Rapid Diagnosis of Pneumonia and Meningitis caused by Chlamydia psittaci

Reviewer's code: 05929462

Position: Peer Reviewer

Academic degree: PhD

Professional title: Research Assistant Professor

Reviewer's Country/Territory: China

Author's Country/Territory: China

Manuscript submission date: 2021-02-25

Reviewer chosen by: Ya-Juan Ma

Reviewer accepted review: 2021-03-19 02:06

Reviewer performed review: 2021-03-21 03:04

Review time: 2 Days

Scientific quality	[Y] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[Y] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection
Re-review	[ ] Yes [Y] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The manuscript "Clinical Metagenomic Sequencing for Rapid Diagnosis of Pneumonia and Meningitis caused by Chlamydia psittaci" by Yin et al. reports a diagnosis method, metagenomics next generation sequencing (mNGS), for rapidly determining pathogens in clinical human samples, including bronchoalveolar lavage fluid and cerebrospinal fluid. It is interesting to see bacterial species other than those typical meningitis-causing bacteria identified in CSF. My major concern is that because C. psittaci was detected along with S. pneumonia, using mNGS in the CSF of the patient with meningitis. It is known that S. pneumonia can cause meningitis and induce inflammation that disrupts the blood-brain barrier. The authors should be more careful to make a conclusion on meningitis caused by Chlamydia psittaci.

Authors' reply:

We thank the reviewer for the valuable comments and acknowledge that *S. pneumonia can cause meningitis* and induce BBB disruption[1, 2]. For Patient 5, mNGS results revealed the co-occurrence of *C. psittaci* and *S. pneumoniae* in the CSF specimen, while the genome coverage rate of *C. psittaci* (7.76%) was much higher than that of *S. pneumoniae* (0.73%) indicative of a higher pathogen load of *C. psittaci*. The patient fully recovered following the anti-*Chlamydia* treatment, whereas it did not exclude possible co-infection. This observation underscored the complexity of diseases related to microbial infections and highlighted the importance of treating patients using personalized anti-infective drug combinations. We have revised the manuscript to bring up this point in Discussion (see lines 229-235).

Minor comments :

--lines 115-116, the softwares used for quality control of the sequencing reads.

Authors' reply:

Trimmomatic v.0.36 software[3] was used to generate high-quality sequencing data by removing low quality reads, adapter contamination, duplicated and short (length <36 bp) reads. We have updated the manuscript accordingly (see line 119).

--lines 118-120, which method was used to assign the filtered reads to the microbial genome database?

Authors' reply:

Kraken 2 (v2.0.7) software[4] was used to assign nonhuman sequences to the microbial genome database, which contains more than 20,000 genome sequences of bacteria, fungi, virus, and parasites for sequence alignment (ftp://ftp.ncbi.nlm.nih.gov/genomes/genbank/). We have updated the manuscript accordingly (see line 122).

Reference

1 Iovino F, Orihuela CJ, Moorlag HE, Molema G, Bijlsma JJ. Interactions between blood-borne Streptococcus pneumoniae and the blood-brain barrier preceding meningitis. PLoS One. 2013: e68408 [PMID: 23874613 10.1371/journal.pone.0068408: 10.1371/journal.pone.0068408]

2 van Sorge NM, Doran KS. Defense at the border: the blood-brain barrier versus bacterial foreigners. Future Microbiol. 2012: 383 [PMID: 22393891 10.2217/fmb.12.1: 10.2217/fmb.12.1]

3 Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014: 2114 [PMID: 24695404 10.1093/bioinformatics/btu170: 10.1093/bioinformatics/btu170]

4 Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. Genome Biol. 2019: 257 [PMID: 31779668 10.1186/s13059-019-1891-0: 10.1186/s13059-019-1891-0]