Reviewer Report

Title: Evaluating the Genome and Resistome of Extensively Drug-Resistant Klebsiella pneumoniae using Native DNA and RNA Nanopore Sequencing

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Reviewer Comments to Author:

The manuscript by Pitt et al interrogated the genome and transcriptome of PDR and XDR K. pneumoniae isolates using the Oxford Nanopore MinION device. This is the very first study which adopted nanopore approaches in direct bacterial mRNA sequencing. The authors established a methodology for adding poly(A) tail onto mRNA transcripts which will benefit future bacterial sequencing and diagnosis related studies. However, authors failed to explain clearly the advantage of using Nanopore for RNA sequencing to Illumina platform. In another word, why we need to develop RNA sequencing using Nanorpore since it is not an efficient way to do it and very complicated. In addition, the manuscript indeed showed that the coverage of RNA seq is very low and the correlation is not good. In my view, if there is no specific need to do RNA seq using Nanoproe platform, there is no need to develop it since the Illumina platform is very good already in this application. In addition, I also have the following major comments:
1. Line 169, section "Antibiotic resistance and the location of acquired resistance in the genome"
The authors reported the AMR genes and their location in this section. Since this is a technical manuscript, can the authors provide some sequencing information? The volume of data generated with time, coverage of each sequenced sample, the accuracy of the sequence, and the comparison of different assembly methods could be briefly discussed.
2. Line 256, only a low proportion of these RNA sequencing reads passed base-calling. Is it also related to the sample preparation apart from the inaccuracy of the base-calling software?
3. Would the authors compare the genome and transcriptome a little bit to link these data?
4. Line 381, "a number of resistance genes were identified that were not present in the final assembly. "The authors were expected to discuss why this happens and how to deal with these false positive data.

Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Choose an item.

Conclusions

Are the conclusions adequately supported by the data shown? Choose an item.

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