Reviewer Report

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

Version: Revision 1 **Date:** 11/6/2019

Reviewer name: Rachael Workman

Reviewer Comments to Author:

The authors' response to our comments and suggestions has improved this manuscript. We are generally satisfied with the manuscript edits and improvements; however, there are a few more modifications, particularly in the presentation of results, that we feel are required to make this paper acceptable for publishing.

1. The response to pt 1 - (why dRNA) is still largely unsubstantiated. The library prep time being cut in half is a slight advantage, but with cDNA we can start from less material (i.e. perform PCR). Multioperon sequencing could be shown from cDNA as easily as from dRNA - and the authors still haven't shown it. And the authors suggest RNA modifications could be detected, arguably the most unique advantage to dRNA seq in prokaryotes - but showed none of this. We recommend rewording rationale to avoid discouraging cDNA sequencing, especially since it is more practical for most applications at this point - and since the promised improvements for dRNA have yet failed to materialize from ONT, and cDNA yield is currently _substantially_ better.
2. In the "levels of expression of resistance genes" section, lines 270-274, it would be useful to include read counts alongside percentages aligned to increase transparency for counts of reads included in the analysis.
3. In line 281 the authors state "These results reflect the fact that base-calling algorithms have not 282 yet been optimised for direct RNA sequencing, and even less so for bacterial RNA sequencing". However, the accuracies reported in the line above are not atypical of single molecule sequencing, and low alignment % seems largely driven by 400-700nt poly-A tails added- recommend amending this statement.
4. More importantly, the alignment % is still alarmingly low - even with current RNA basecallers you should be seeing 80-90% alignment. We recommend filtering reads before alignment by only using "pass" reads, trimming poly-A tails off reads, and removing small reads less than 75nt (which are more likely to be noise), then reporting this alignment percentage.
5. Figure 3- Please denote on the figure itself which primers recognize more than one gene, maybe with underlining? Also, what does the +0.001 notation on axes mean?
6. Figure 5 is overly complicated with the shapes and colors and asterisks - why not just plot the data in the same way as Figure 3, and you can facet by sample?

Small things:

Spell out XDR first (line 329)

Please parse paragraph lines 348-388 into DNA and RNA sections

Methods

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

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R.E.W. and W.T. received reimbursements for travel, accommodation and conference fees to speak at events organised by Oxford Nanopore. W.T. has two patents (8,748,091 and 8,394,584) licensed to ONT

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