Reviewer's report

Title: Analysis on Porin I genotype diversities and correlation of gene mutations and drug resistance in Neisseria gonorrhoeae isolates in Eastern China

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Reviewer: Lori Snyder

Reviewer's report:

In this work the authors have examined the gene sequences, and therefore predicted protein sequences, of the alleles of the gene encoding PI from a collection of clinical isolates from Eastern China. They have designed primers to amplify the whole gene and to amplify the PIA and PIB alleles of the gene in such a way that they are distinguishable on a gel. Some new mutations are described and conclusions are drawn about associations of these mutations with resistance to penicillin and tetracycline.

• Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

1. The research question posed here and/or the hypothesis being tested in not very clear. It needs to be made clearer to the reader what the goals of this study were. This will also help the reviewer and the editor decide if these goals have been met.

2. The nomenclature used to differentiate the different porin types is possibly not the most commonly used / up to date version, i.e. PI, PII, and PIII, and therefore other terminology, like PorA and PorB, might be clearer to the reader. The authors should review the literature and use the terminology that is most commonly used by researchers in the field today.

3. There have been reports of strains of N. gonorrhoeae that react to both PIA and PIB antibodies (De La Fuente and Va´zquez, Sex Transm Dis, 1991; Gill et al., FEMS Microbiol Lett, 1994). This has been presumed to be due to mosaicism between porB1a and porB1b alleles of the gene. This is not mentioned in the Background and there is no discussion of mosaicism in the Results or Discussion. If none was observed, this should be mentioned. If it was observed, this should be discussed.

4. Even using a proof-reading enzyme, errors might be introduced into the sequence through the PCR amplification process. This is not insurmountable when the PCR product is then directly sequenced, since the error is likely to be visible in the base-by-base fluorescence peak analysis. In this work, however, the PCR products have been cloned and just one of the pool of PCR products has been selected within the clone – potentially freezing and preserving an error that occurred in PCR. The justification posed by the authors for cloning the PCR products was “to obtain more accurate sequencing data”. This is not the case.
due to the nature of PCR and the selection that occurs in cloning.

5. There is no information in the Methods section on the sequencing methods used or the analysis of the sequence data information. It is important to know if this was double-stranded sequencing (i.e. from both ends), the length of the sequence read (i.e. long enough for the whole gene), how any N’s or dye blobs were resolved, whether there were any ambiguous bases, and whether any internal primers were needed to finish the sequence.

6. There are minor grammatical errors throughout that must be addressed, but are too numerous to list here. In addition, word usage should be confirmed to be correct, for example “revise” is used on page 6 when the intended word is probably “reverse”. Also there are some stray italics on page 8.

7. The Results section of the Abstract is quite difficult to follow and seems not very dissimilar to the text on page 8 and 9. The Abstract should be re-written to be more easily understood and in a way that attracts the reader so that they will want to read the whole article.

8. Please address the following questions about the Methods. Why in the duplex PCR was 15 pmol of primer pIA/B-D-F2 used when the other 2 primers were used at 20 pmol concentrations? Also, why did the PCR reaction temperature and cycling conditions change in the duplex PCR from the normal PCR?

9. It is difficult to categorically draw any conclusions about the influence of point mutations on antimicrobial resistance without the construction of isogenic mutants. The authors should be careful not to overstate discoveries of mutations in clinical isolates that also happen to be resistant to certain antibiotics before a direct association can be determined. In light of the nature of the data upon which the authors are trying to draw this conclusion, I do not believe it should be such a focus of the manuscript or that it should be so prominently in the title.

10. The reader is left wondering about the nature of this isolate collection. What other information is available about these isolates from the clinical laboratories that isolated and classified them? Was porin typing by antibodies or other methods done? Was antibiotic resistance determined? Certainly other data must be available from the hospital. Since N. meningitidis strains have been found that contain PIB (Va´zquez et al., Mol Microbiol, 1995), were any tests conducted within your laboratory to confirm the classification of these isolates as N. gonorrhoeae.

- Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
  None at this time.

- Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)
  None at this time.
Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests