Reviewer's report

Title: Detection of virulence genes in Malaysian Shigella spp. strains by multiplex PCR assay

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Reviewer: Martin Altwegg

Reviewer's report:

In this manuscript the development of a multiplex PCR assay (mPCR) for the simultaneous detection of various virulence-associated genes in Shigella species is described. As presented, the four genes are amplified with about equal efficiency except for the amplicon with the smallest size (147bp) indicating that optimization has been done carefully.

Compulsory revisions:

1. extensive modifications in the manuscript with respect to the following considerations (does not need further experiments:
   Shigellae (genetically belonging to the species Escherichia coli) are a group of organisms that can be differentiated from other bacteria based on biochemical and serologic criteria and not on the basis of virulence factors that may be present or absent.
   The four virulence-associated targets (set1A and set1B located on the chromosome; ial located on the virulence plasmid; ipaH located on both the plasmid and the chromosome). While the enterotoxin genes seem to be present mainly in Shigella flexneri 2a, the other serotypes of this species as well as the other 3 species are nevertheless considered pathogenic for humans but may be associated with a different clinical presentation (less severe infection?). Thus, the clinical utility of a test targeting these two genes is limited.
   The problem of the ial locus is that it is located on the plasmid that may be rapidly lost entirely by repeated subculturing. In addition, partial deletions may also explain why PCR becomes negative. As above, the absence of ial does not indicate that a bacterium is not a Shigella sp., although it may indicate that a strain is not pathogenic anymore. However, this does not necessarily mean that the patient did not suffer from shigellosis as the loss of the plasmid may have occurred just in the primary culture. For the present study strains that were several years old have been used. It has previously been shown that the frequency of a positive ial-PCR is much lower in strains stored for years than in fresh clinical specimens (reference 8 showed a good correlation of ial and ipaH in fresh clinical specimens).
   Thus, ipaH seems the target of choice because it is present on both the plasmid and the chromosome. However, nothing can be said about the virulence of a ipaH-positive strain. In this respect, this parameter fits best with the standard method which is culture.
   With above in mind, I doubt that a mPCR gives more valuable clinical information than a ipaH PCR alone. In addition, no clue is given on what to do with conflicting results, e.g. ipaH-positive, set-genes negative or ial negative. A useful algorithm for rapid results might be to do ipaH-PCR. If
this is positive, presumptive presence of Shigella/enteroinvasive E.coli could be reported and culture on selective media be performed.

The exclusion of enteroinvasive E. coli from the study is very arbitrary.

2. Sensitivity testing of mPCR
The first problem with regard to sensitivity is the fact that cfu determinations have been determined using SS agar rather than blood agar. It is well known that selective media may have a significantly lower plating efficiency than a nutrient-rich non-selective agar. Thus, the number of organisms present in the suspensions used is most probably higher than the numbers given in the manuscript.
Secondly, the sensitivity of the mPCR is given in cfu determined prior to an enrichment step in BHI. This is certainly misleading and should be avoided.

Minor revisions:

1. In the M+M section optimization experiments to establish the mPCR should be mentioned and some more information about the results of these experiments should be given (bottom of page 7).

2. Specificity testing is not really sufficient with only 12 species having been tested. Stool specimens from healthy people should be available without much effort.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

None