Author's response to reviews

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

Authors:

Kwai-Lin Thong (thongkl@um.edu.my)
Susan ling ling Hoe (susanllhoe@yahoo.com)
Savithri D. Puthucheary (puthu@um.edu.my)
Rohani MD. Yasin (rohanip@imr.gov.my)

Version: Date: 28 October 2004

Author’s response to reviews:

To The BioMed Central Editorial

Dear Sir

I am resubmitting a revised manuscript entitled Detection of virulence genes in Malaysian Shigella spp by multiplex PCR assay

Thank you

Dr Thong

Response to the Referees:

Response to First Reviewer: S.K.Bhattacharya

1. A brief description of the relevant virulence genes is given in page 3, lines 8-22

2. Source of antisera listed in Methods (page 3, line 34)

3. I agree with the comment of the reviewer regarding the specificity of the assay in the absence of the testing with EIEC strains. Hence the word 'specificity' of the assay has been removed from the revised manuscript

Specific comments

1. PCR on direct cell lysate would react to both chromosomal and plasmid DNA. Hence, one possibility of the absence of plasmid-borne genes such as ial would indicate loss of plasmid -as described in the later part of the Discussion (page 7, line 21-27)

2. Comments noted. Design of newer primers would be recommended for future work

3. BHI was used for dilution of the fecal sample. It is the dilution and preincubation that help to reduce the inhibitors. The word 'background flora' has been reduced in the revised manuscript. In addition two sentences were added to explain this. Page 7, lines 33-41 of the Revised Manuscript.

Dilution of the faecal sample with BHI was performed to lower the levels of PCR inhibitors such as bilirubin, bile salts and heme in the faeces [16]. An additional step of preincubating the spiked faecal samples also
helped to eliminate the natural inhibitors [24]. The short 4-h enrichment step would increase the total number of target sequences caused by more bacterial growth and the overall detection sensitivity of the assay. Although PCR cannot differentiate between dead and viable bacteria, enrichment helped to dilute the concentrations of dead bacteria, thus reducing the probability of detecting them by the subsequent mPCR assay.

Response to Second Reviewer Lei Wang

General Comments

1. I agree with the Reviewer that it is inappropriate to say that the PCR assay is specific for Shigella only as we did not test the EIEC strains. Hence, we have removed the word `specific' throughout the manuscript. We also modify the conclusions based on the data obtained.

2. We initially tried to incorporate all the 5 virulent genes, including sen, However, in spite of repeated optimizations and experiments, sen gene could not be co-amplified with the other 4 genes. Hence, we performed the multiplex on the 4 genes as described in the manuscript and a monoplex PCR for sen only. We present the data on the multiplex PCR assay only.

Specific comments

1. The conclusions have been modified according to the Reviewer's recommendation: We delete the word 'specific'.

In the Abstract page 2: the mPCR system is reproducible, sensitive and is able to identify pathogenic strains of Shigellae irrespective of the locality of the virulence genes. It can be easily performed with a high throughput to give a presumptive identification of the causal pathogen.

Also, on page 8, lines 13-19 (Revised Manuscript): We conclude that the mPCR system is able to identify pathogenic strains of Shigellae irrespective of the locality of the virulence genes. The described assay is reproducible, sensitive, easy to perform with a high throughput and is able to give a presumptive identification of the causal pathogen, which should be confirmed by culture techniques using selective media. An added advantage would be that EIEC, which gives a similar illness, may also be detected by this method, as EIEC also harbours ial and ipaH genes.

2. Rephrase the sentence page 8,line 8-9 : Our study supported the observations of Noriega et al. [9] and Vargas et al. [17] in that our Shigella strains, both set1A and Set1B were present exclusively in S.flexneri 2a.

3. same response as in #1

Response to third Reviewer Prof Martin Altwegg

1. We agree with the Reviewer's comments that the differentiation of the Shigella spp from other Enterobacteriae is based on the biochemical and serologic criteria - a statement added in the conclusion alluding to this point ( page 8, line 14-16).

The main objective of the study was to determine the prevalence of the commonly reported virulence associated genes in the Malaysian Shigella spp rather than identification of the species. As this is the first study being carried out in our local strains, we have no prior knowledge of the presence/absence of these genes. Based on the study, we agreed with the Reviewer that ipaH PCR alone would be useful as it is
present in 100% of the strains studied, irrespective of the serotype. As suggested by another Reviewer, designing of a serotype specific primers based on the 16S rDNA sequences would be considered for future work.

Other comments:

1. Sensitivity testing of mPCR

We added a phrase after quoting the sensitivity value to indicate that the cfu cited is before the short incubation prior to PCR analysis of the fecal samples.

The mPCR could detect 100 colony-forming units (cfu) of shigellae per reaction mixture in spiked faeces following a 4h-preincubation.

A good point raised by the Reviewer regarding the use of SS agar and the lower number of cells growth. In addition to SS agar, plating was also done on MacConkey and LB agar but not on blood agar.

1. In the M+M, we elaborated the optimization experiments (page 3 line 43-page 4,line 14) and added more results on page page 5, line 17-39

2. Comments noted. Future work will include more stool specimens

Dr Thong Kwai Lin
Corresponding author