Reviewer's report

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

Version: 2 Date: 8 September 2004

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This study by Hoe et al describes about the multiplex PCR assay designed from published works and standardized for the detection of virulence genes either from pure cultures or with stool enrichment cultures. The manuscript was well written with clear description of the study. The methods used in this study were appropriate.

General comments:

1. In the background section of the manuscript, a brief description of different virulence genes associated with shigellosis can be mentioned

2. Page 3, lines 21. Name of the antisera supplier was not mentioned

3. Since the EIEC also harbours ial and ipaH genes, the specificity of the stool enrichment PCR for the detection of Shigella spp is not dependable. The interpretation of ial as well as ipaH genes should be considered with caution in the mPCR.

Specific comments:

1. Page 9, line 17-18. In the mPCR assay, the virulence attribute of the suspected strain does not specify to its chromosome or plasmid if the culture lysates are used (as shown in the material and methods) as the mPCR would react both with chromosomal and plasmid DNA.

2. The use of set genes in the mPCR assay draws less attention, as the genes are conserved for certain serotypes of S. flexneri. Instead, the authors would have tried to design primers, which are species specific from 16S rDNA sequences either of Shigella spp or EIEC to increase the utility of the mPCR.

3. Page 11, line 15. Use of BHI in the enrichment is step is a good choice, but no microbiologist will agree that the BHI has the potential to reduce the background flora if stool specimens were inoculated.