CHAPTER 5

CONCLUSION

All the objectives of research had been achieved. Boiling method was found to be more efficient than phenol-chloroform extraction method in producing suitable DNAs for amplification. Sequencing of the amplicons found that all of them had more than 95% sequence similarities with the published sequences. Reproducibility of four genes co-detected in the mPCR was 100% compared to 78.6% of sen amplification in the monoplex PCR. The amplification systems developed in this study were 100% specific for Shigella spp.; however, it had been reported that ial, ipaH and sen were also found in EIEC strains that harbour the inv plasmid.

Both mPCR and monoplex PCR were used to detect the presence of five virulence-associated genes in 110 strains of Shigella spp. from the IMR. All the strains tested positive for chromosomal-encoded setLA and setLB genes showed 100% correlation in the presence of both genes. Their almost exclusive prevalence in S. flexneri 2a was proven to be statistically significant in the Malaysian collection, as reported in other studies. The other virulence-associated genes, ial, ipaH and sen were more well spread. The instability of the large inv plasmid was postulated to affect the prevalence of plasmid-encoded ial and sen. The location of ial gene cluster seemed to be less influenced by selective deletion events than the sen region on the plasmid, judging by its higher prevalence than sen. Another plasmid-encoded gene, ipaH is less compromised by plasmid loss or deletions because it is also located on the Shigella chromosome. All 110 strains yielded a presence for it. Nevertheless, its sole presence in Shigella and EIEC strains cannot be regarded as an indicator of invasiveness as it had been shown to hybridize to strains that had lost the inv plasmid in a previous study. Hence, in the detection of invasive shigellae, ipaH must be combined with at least another plasmid-encoded gene.
Judging from its efficacy in bacterial strains and spiked faeces, the mPCR has the potential to be refined, developed and employed as a powerful molecular detection tool in routine diagnosis of shigellosis in clinical centers as well as in epidemiological studies.