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 *set1A* and *set1B* 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.