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**DEVELOPMENT OF A MULTIPLEX PCR IN THE RAPID DETECTION OF
VIRULENCE-ASSOCIATED GENES IN *Shigella* spp.**

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ABSTRACT

Current practice of culturing and isolating *Shigella* spp. from stool specimens in order to identify the specific bacterium agent responsible for bacillary dysentery or shigellosis by biochemical tests, has often met with limited success. In this study, a more rapid and specific means of identification had been demonstrated via a multiplex PCR (mPCR) for the simultaneous detection of both chromosomal- and plasmid-encoded virulence-associated genes (*set1A*, *set1B*, *ial* and *ipaH*) and a monoplex PCR for the separate detection of *sen* gene. One hundred and ten strains of Malaysian *Shigella* spp. isolated from years 1997 to 2000 were tested. Boiling method was used to prepare DNA templates for PCRs. *ipaH* was present in all the local isolates, while both *set1A* and *set1B*, *ial* and *sen* were identified in 40.9%, 40.9% and 30.9% of the strains respectively. The tandem chromosomal genes of *set1A* and *set1B* were primarily detected in *S. flexneri* 2a strains, *sen* gene was found in all *Shigella* species except for *S. sonnei* and both *ial* and *ipaH* were more widespread in all four species. The two most frequent pathotype profiles shown were the presence of *ipaH* (34/110 strains) and the presence of *set1B/set1A/ipaH* (21/110 strains). Both the amplification assays were specific, as non-*Shigella* strains did not generate any desired PCR product. The average detection sensitivity limit of the mPCR in brain heart infusion (BHI)-preincubated *S. flexneri* 2a-spiked faeces was approximately 5.0×10^4 cfu/ml or 100 cfu of shigellae per mPCR reaction and is within the common infectious dose of at least 10^4 viable cells. Strains of *Shigella* spp., regardless of the location of the virulence-associated genes, could be rapidly detected in a single assay, hence hastening appropriate medical treatment of patients.

ABSTRAK

Pengkulturan and isolasi *Shigella* spp. pada masa kini dari spesimen tinja untuk identifikasi agen bakteria spesifik yang menyebabkan disentri basilari atau shigellosis, melalui ujian biokimia, selalunya mempunyai kejayaan terhad. Dalam kajian ini, cara yang lebih cepat and spesifik untuk identifikasi telah dibuktikan melalui tindakbalas rantaian polimeras multipleks (mPCR). Tindakbalas ini adalah untuk deteksi serentak gen-gen virulen (*set1A*, *set1B*, *ial* dan *ipaH*) yang dikodkan oleh kromosom serta oleh plasmid. Deteksi gen *sen* dijalankan berasingan dalam satu tindakbalas rantaian polimeras monopleks. Sebanyak 110 stren *Shigella* spp. Malaysia yang diisolasi dari tahun 1997 hingga 2000 telah dikaji. Kaedah pendidihan digunakan bagi menyediakan templat DNA untuk PCR. *ipaH* hadir dalam semua isolasi, manakala kedua-dua *set1A* dan *set1B*, *ial* dan *sen* ditemui dalam 40.9%, 40.9% dan 30.9% daripada stren-stren masing-masing. Gen-gen kromosom tandem *set1A* dan *set1B* dikesan dalam stren *S. flexneri* 2a, gen *sen* pula dalam semua spesies *Shigella* kecuali *S. sonnei* dan kedua-dua *ial* dan *ipaH* adalah lebih meluas dalam keempat-empat spesies. Profil patotip ("pathotype") yang paling kerap ditunjukkan ialah kehadiran *ipaH* (34/110 stren) dan kehadiran *set1B/set1A/ipaH* (21/110 stren). Kedua-dua esei amplifikasi adalah spesifik kerana stren bukan *Shigella* tidak mengenerasikan produk PCR yang dikehendaki. Purata had sensitiviti mPCR dalam tinja yang diinokulasi dengan *S. flexneri* 2a dan diinkubasi dalam brain heart infusion (BHI) adalah kira-kira 5.0×10^4 cfu/ml atau 100 cfu shigellae per mPCR. Ini adalah di dalam julat dos infeksi biasa shigellosis iaitu 10^4 sel yang hidup ("viable"). Stren-stren *Shigella* spp. tidak kira lokasi gen virulennya, boleh dikesan dengan cepat dalam satu esei supaya pemberian ubat yang bersesuaian boleh dipercepatkan.

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TABLE OF CONTENTS

TITLE	i
ABSTRACT	ii
ABSTRAK	iii
ACKNOWLEDGEMENT	iv
TABLE OF CONTENTS	v
LIST OF FIGURES	ix
LIST OF TABLES	x
LIST OF SYMBOLS AND ABBREVIATIONS	xii
CHAPTER 1: INTRODUCTION	
1.1 The genus <i>Shigella</i>	1
1.2 Clinical manifestation	2
1.3 Epidemiology	3
1.4 Pathogenesis and virulence-associated factors	
1.4.1 Pathogenesis	4
1.4.2 Virulence-associated factors	
1.4.2.1 Plasmid-encoded	7
1.4.2.2 Chromosomal-encoded	9
1.4.2.3 Pathogenicity islands	10
1.5 Laboratory diagnosis	
1.5.1 Culture examination	11
1.5.2 Biochemical identification	12
1.5.3 Serotyping	12
1.6 Polymerase chain reaction (PCR)	13
1.6.1 The PCR process	
1.6.1.1 DNA denaturation	14
1.6.1.2 Annealing of primer to target	14
1.6.1.3 Extension of target sequences	14
1.6.2 Types of PCR-based amplification techniques	17
1.6.3 Applications of PCR in the study of <i>Shigella</i> spp.	18
1.7 Multiplex PCR	
1.7.1 Reaction components	19
1.7.1.1 Oligonucleotide primers	20
1.7.1.2 PCR buffer	21
1.7.1.3 Magnesium chloride	21
1.7.1.4 Deoxynucleotides	22
1.7.1.5 <i>Taq</i> DNA polymerase concentration	22
1.7.1.6 PCR additives	24
1.7.1.7 DNA template	24
1.7.1.8 Oil overlay	25

1.7.2	Reaction conditions	
1.7.2.1	Denaturation	26
1.7.2.2	Annealing	26
1.7.2.3	Extension	27
1.7.2.4	Number of cycles	27
1.7.2.5	Automation	27
1.7.3	Contamination of mPCR	28
1.7.4	Applications	31
1.8	Treatment and prevention	
1.8.1	Treatment	31
1.8.2	Prevention	33
1.9	Rationale for study	34
1.10	Objectives of study	35

CHAPTER 2: MATERIALS AND METHODOLOGY

2.1	Materials	
2.1.1	Bacterial strains	36
2.1.2	Clinical specimens	36
2.1.3	Chemicals	36
2.1.4	Media for bacteria growth	
2.1.4.1	Luria-Bertani (LB) broth	38
2.1.4.2	LB agar	38
2.1.5	Medium for bacteria enrichment	
2.1.5.1	Brain Heart Infusion (BHI)	38
2.1.6	Media for bacteria selection	
2.1.6.1	MacConkey agar	39
2.1.6.2	<i>Salmonella Shigella</i> (SS) agar	39
2.1.7	Solutions for phenol-chloroform DNA extraction	
2.1.7.1	0.5M EDTA, pH 8.0	39
2.1.7.2	0.15M NaCl, 0.1M EDTA, pH 8.0	40
2.1.7.3	1M Tris-HCl, pH 8.0	40
2.1.7.4	Lysis buffer (1% SDS, 0.1M NaCl, 0.1M Tris-HCl, pH 8.0)	40
2.1.7.5	5M sodium perchlorate	40
2.1.7.6	RNase A (10 mg/ml)	40
2.1.8	Solutions for agarose gel electrophoresis and gel visualization	
2.1.8.1	10X Tris-borate EDTA (TBE) buffer	41
2.1.8.2	2% agarose gel	41
2.1.8.3	Ethidium bromide (10 mg/ml)	41
2.1.9	Oligonucleotide primers	42
2.2	Methodology	
2.2.1	Sterilization techniques	
2.2.1.1	Moist heat	43
2.2.1.2	Membrane sterilization	43
2.2.1.3	UV irradiation	43
2.2.2	DNA extraction methods	
2.2.2.1	Boiling	45
2.2.2.2	Phenol-chloroform extraction	45

2.2.3	Polymerase chain reaction (PCR)	
2.2.3.1	Optimization of monoplex PCR	46
2.2.3.2	Optimization of multiplex PCR (mPCR)	48
2.2.3.3	Purification of PCR products	49
2.2.3.4	Sequencing	50
2.2.3.5	Reproducibility	50
2.2.3.6	Specificity	50
2.2.3.7	Sensitivity	51
2.2.3.8	Faecal-spiking and sensitivity	51
2.2.3.9	Screening of clinical specimens	52
2.2.3.10	Agarose gel electrophoresis	52
2.2.3.11	Gel visualization and documentation	53
2.3	Data analyses	53

CHAPTER 3: RESULTS

3.1	Comparison of DNA extraction methods	55
3.2	Optimization of monoplex PCRs	57
3.3	Optimization of multiplex PCR (mPCR)	58
3.3.1	Different primer concentrations	59
3.3.2	Different buffer concentrations	61
3.3.3	Different annealing temperatures	62
3.3.4	Different <i>Taq</i> DNA polymerase concentrations	63
3.3.5	Different dNTPs concentrations	64
3.4	Sequencing	66
3.5	Reproducibility	68
3.6	Specificity	69
3.7	Sensitivity	70
3.8	Faecal-spiking and sensitivity	71
3.9	Prevalence of virulence-associated genes in Malaysian strains	72
3.9.1	Distribution of virulence-associated genes according to species and serotype	74
3.9.2	Distribution of virulence-associated genes according to year of isolation	76
3.9.3	Analysis of the profiles of virulence markers (pathotypes) in Malaysian <i>Shigella</i> spp.	78
3.10	Clinical specimens	79

CHAPTER 4: DISCUSSION

4.1	General	80
4.2	Comparison of DNA extraction methods	81
4.3	Optimization processes	
4.3.1	Monoplex PCR	83
4.3.2	Multiplex PCR (mPCR)	85
4.4	Sequencing	89
4.5	Reproducibility	91
4.6	Specificity	91
4.7	Sensitivity	92
4.8	Faecal-spiking and sensitivity	93

4.9 Prevalence of virulence-associated genes in Malaysian strains	97
4.9.1 Overall prevalence of virulence-associated genes	98
4.9.2 Distribution of virulence-associated genes according to species and serotype	99
4.9.3 Distribution of virulence-associated genes according to year of isolation	102
4.9.4 Analysis of the profiles of virulence markers (pathotypes) in Malaysian <i>Shigella</i> spp.	103
4.10 Clinical specimens	104
4.11 Implications of this study	105
4.12 Limitations of the present study	106
 CHAPTER 5: CONCLUSION	 107
 BIBLIOGRAPHY	 109
 APPENDIX 1: DETAILS OF THE 110 MALAYSIAN <i>Shigella</i> STRAINS USED IN THIS STUDY	 122
 APPENDIX 2: STANDARD NUCLEOTIDE-NUCLEOTIDE BLAST SEARCH RESULTS	 127

LIST OF FIGURES

	<u>Title</u>	<u>page</u>
Fig 1.1	Entry and dissemination of <i>Shigella</i> in epithelial cells <i>in vitro</i>	6
Fig 1.2	Polymerase chain reaction (PCR)	16
Fig 2.1	General experimental steps	44
Fig 3.1	Amplicons generated from DNAs prepared by the boiling and phenol-chloroform extraction methods	56
Fig 3.2	Optimized multiplex PCR and mPCR	57
Fig 3.3	Amplicons generated by a combination of 5 primer sets at an equimolar concentration of 0.5 μ M each in mPCR	58
Fig 3.4	Optimization of primer concentrations in mPCR	59
Fig 3.5	Further optimization of primer concentrations in mPCR	60
Fig 3.6	Optimization of buffer concentration in mPCR	61
Fig 3.7	Optimization of annealing temperature in mPCR	62
Fig 3.8	Optimization of <i>Taq</i> DNA polymerase concentration in mPCR	63
Fig 3.9	Optimization of dNTPs concentration in mPCR	64
Fig 3.10	Polymorphic site(s) within the virulence-associated genes in <i>Shigella</i> spp.	67
Fig 3.11	Specificity results of mPCR and multiplex PCR	69
Fig 3.12	Sensitivity result of mPCR assay by using DNA templates from bacterial culture at different dilutions	70
Fig 3.13	Faecal-spiking and sensitivity result of mPCR (without additional BHI)	71
Fig 3.14	Faecal-spiking and sensitivity result of mPCR (with additional BHI)	72

LIST OF TABLES

	<u>Title</u>	<u>page</u>
Table 1.1	Selected biochemical reactions of typical <i>Shigella</i> isolates	12
Table 1.2	Examples of various PCR-based amplification techniques in the detection of microorganisms or specific genes	18
Table 1.3	Examples of mPCR applications	31
Table 2.1	Chemicals and reagents used in this study	37
Table 2.2	Primer sets used for identification of virulence-associated genes in <i>Shigella</i> spp.	42
Table 2.3	Reaction mixture for the optimization of monoplex PCR	47
Table 2.4	Reaction mixture for the initial attempts of mPCR optimization (incorporating 10 primers)	48
Table 2.5	Reaction mixture for the optimization of mPCR (incorporating 8 primers)	49
Table 3.1	Prevalence of virulence-associated genes in DNAs prepared by the boiling and phenol-chloroform extraction methods	56
Table 3.2	Reproducibility of amplification results	68
Table 3.3	Prevalence of all the virulence-associated genes in Malaysian <i>Shigella</i> spp.	73
Table 3.4	Paired samples t-test results	74
Table 3.5	Prevalence of virulence-associated genes according to species	75
Table 3.6	Chi-square test results for the determination of a significant association between the prevalence of virulence-associated genes and <i>S. flexneri</i> 2a serotype	76
Table 3.7	Prevalence of virulence-associated genes according to year of isolation	77

	<u>Title</u>	<u>page</u>
Table 3.8	Comparison of prevalence of <i>ial</i> and <i>sen</i> with year of isolation	78
Table 3.9	Pathotypes of <i>Shigella</i> spp. based on the presence of virulence-associated genes	79

LIST OF SYMBOLS AND ABBREVIATIONS

~	approximately
χ^2	chi-square
$^{\circ}\text{C}$	degrees Celsius
=	equals to
<	less than
>	more than
μl	microlitre
μg	microgram
μM	micromolar
%	percent
α	significance level
H_a	alternative hypothesis
H_o	null hypothesis
A	adenine
ATCC	American Type Culture Collection
bp	base pair
BHI	Brain Heart Infusion
cm	centimetre
cfu	colony forming unit
cfu/ml	colony forming unit per millimeter
C	cytosine
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide
dH ₂ O	distilled water
ddH ₂ O	deionized distilled water

EDTA	ethylenediaminetetraacetic acid
EIEC	enteroinvasive <i>Escherichia coli</i>
Fig	Figure
g	gram
G	guanine
h	hour
IMR	Institute for Medical Research
<i>ial</i>	invasion-associated locus
<i>ipa</i>	invasion plasmid antigen
kb	kilobase
kDa	kiloDalton
LB	Luria-Bertani
MgCl ₂	magnesium chloride
M	molar
min	minute
mM	millimolar
ml	millilitre
mm	millimetre
mPCR	multiplex PCR
NaCl	sodium chloride
Ng	nanogram
nm	nanometre
PAI	pathogenicity island
PCR	polymerase chain reaction
PCR-ELISA	PCR-enzyme-linked immunosorbent assay
PCR-SSCP	PCR-single-strand conformation polymorphism

p.s.i	pound per square inch
p	probability
RNase	ribonuclease
rRNA	ribosomal ribonucleic acid
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
sec	second
SS	<i>Salmonella-Shigella</i>
T	thymine
TBE	Tris-borate-EDTA
Tris	Tris(hydroxymethyl) methylamine
Tris-HCl	Tris-hydrochloric acid
UV	ultraviolet
U	unit
V	volt
w/v	weight per unit volume
WHO	World Health Organization
XLD	xylose lysine deoxycholate