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 X 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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~	approximately
χ^2	chi-square
^0C	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_2O	distilled water
ddH_2O	deionizied distilled water

EDTA	ethylenediaminetetraacetic acid
EIEC	enteroinvasive *Escherichia coli*
Fig	Figure
g	gram
G	guanine
h	hour
IMR	Institute for Medical Research
ial	invasion-associated locus
ipa	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	*Salmonella-Shigella*
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