ABSTRACT

In this study, *Salmonella enterica* serovar Typhi (*S.* Typhi) strains collected from outbreak and sporadic cases of typhoid fever during the years 1998 to 2007 in Malaysia were characterized using three PCR-based methods, i.e., restricted arbitrarily primed PCR (resAP-PCR), repetitive element PCR (rep-PCR) and variable-number tandem repeat (VNTR) analysis. The objective of this study was to explore the utility of VNTR and resAP-PCR as newer molecular methods in comparison with the commonly used method of rep-PCR to subtype *S.* Typhi. ResAP-PCR analysis was conducted using arbitrary primers STHae8 and STAlu9 following endonuclease digestion of *S.* Typhi DNA with *Hae*III and *Alu*I enzymes. Rep-PCR was performed by using REP-F and REP-R primers, corresponding to the highly conserved REP repeated DNA elements. VNTR analysis was performed by multiplex approach using four primers (TR1, TR2, TR3, TR5) flanking VNTR loci in the CT18 strain of *S.* Typhi.

The *S.* Typhi strains were typeable by three methods in assigning a defined type to each strain tested. Restricted AP-PCR amplification of *Hae*III and *Alu*I digested DNA using STHae8 and STAlu9 primers was not able to discriminate *S.* Typhi strains tested. In addition, resAP-PCR analysis using STHae8 primer demonstrated poor reproducibility. Similarly, rep-PCR with the primers REP-F and REP-R was not useful as a subtyping tool for *S.* Typhi as all strains shared the same predominant banding profile. In contrast, multiplex VNTR assay displayed diversity based on four VNTR loci studied, with total allele numbers ranging from 2 to 19 and Nei's diversity (*D*) values ranging from 0.04 to 0.93. Sequence analysis of individual alleles confirmed the presence of VNTRs among the strains and identified 42 VNTR

profiles. The results revealed that the copy number of repeats in CT18 strain of *S.* Typhi was highly correlated with the *D* value ($R^2 = 0.57$) and number of alleles ($R^2 = 0.87$) observed across diverse strains. The VNTR analysis showed that significant genetic heterogeneity exists at TR1, TR2 and TR3 loci of *S.* Typhi strains in Malaysia. Analysis of the VNTR patterns indicated that *S.* Typhi strains obtained from sporadic cases were much more heterogeneous than those obtained during outbreaks of typhoid fever. The findings in this study demonstrate that VNTR is able to provide a rapid, highly discriminative, and reproducible typing method for epidemiological surveillance and outbreak investigation of *S.* Typhi strains.

ABSTRAK

Salmonella enterica serovar Typhi yang dikaji terdiri dari strain bakteria yang dikumpul dari kes deman typhoid di Malaysia dari tahun 1998 hingga 2007. Strain bakteria ini dikaji dengan menggunakan tiga kaedah PCR iaitu, 'restricted arbitrary primed PCR' (resAP-PCR), 'repetitive element PCR' (rep-PCR), dan 'variable-number tandem repeats' (VNTR). Objektif kajian ini adalah untuk meneroka kegunaan VNTR dan resAP-PCR sebagai kaedah molekul yang baru berbanding dengan kaedah rep-PCR untuk menjeniskan *S.* Typhi. Analisa resAP-PCR dijalankan dengan menggunakan arbitrary primers STHae8 dan STAlu9 dan penghadaman endonuclease amplikon dengan menggunakan enzim *Hae*III dan *Alu*I. Rep-PCR dijalankan dengan menggunakan primer REP-F dan REP-R. Analisa VNTR dijalankan dengan menggunakan pendekatan multiplex mengandungi empat primer iaitu (TR1, TR2, TR3, dan TR5) flanking VNTR loci di strain *S.* Typhi CT18.

Strain-strain *S.* Typhi boleh ditaip dengan 3 kaedah. Amplifikasi AP-PCR pada *Hae*III dan *Alu*I digested DNA dengan menggunakan primer STHae8 dan STAlu9 tidak dapat mendiskriminasi *S.* Typhi yang dikaji. Selain itu, keputusan resAP-PCR ini dengan menggunakan primer STHae8 tidak dapat dihasilkan semula. Analisa rep-PCR dengan menggunakan primer REP-F dan REP-R adalah tidak berguna memandangkan keputusan yang sama diperolehi. Multiplex assay VNTR dapat menunjukkan diversiti *S.* Typhi melalui 4 VNTR loci yang dikaji dengan jumlah allel dari 2-19 dan indeks diversiti Nei's (*D*) dari 0.04-0.93. Analisa sequence pada allel individu mengesahkan kehadiran 42 profil VNTR. Keputusan ini menunjukkan bahawa salinan ulangan nombor di strain *S.* Typhi CT18 mempunyai hubungan yang tinggi dengan nilai *D* (R^2 =

0.57) dan nombor allel (R^2 = 0.87) didapati pada strain. Analisa VNTR menunjukkan bahawa strain-strain *S.* Typhi yang didapati dari kes sporadik adalah amat berbeza berbanding dengan kes outbreak deman typhoid. Kajian ini menunjukkan kaedah VNTR adalah cepat, mempunyai diskriminasi yang tinggi dan boleh ulang semula serta berguna dalam surveillance epidemiologi dan siasatan outbreak *S.* Typhi.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBERIVATIONS

°	Degree
°C	Degree Celsius
µl	Microlitre
µg	Microgram
%	Percent
AFLP	Amplified Fragment Length Polymorphism
AP-PCR	Arbitrarily Primed PCR
bp	base pair
BSA	Bismuth Sulphite Agar
D	Nei's diversity index
ddH_2O	Deionised distilled water
dH_2O	Distilled water
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide Triphosphates
EDTA	Ethylene Diamine Tetraacetic Acid
EtBr	Ethidium Bromide
g	gram
h	hour
HCl	Hydrogen chloride
Kb	Kilobase pair
LB	Luria-Bertani
LPS	Lipopolysaccharide Analysis
M	Molar

MDR	Multidrug-resistant
$MgCl_2$	Magnesium chloride
MLEE	Multilocus Enzyme Electrophoresis
mM	millimolar
mg	miligram
ml	millilitre
mRNA	Messenger Ribonucleic Acid
NaOH	Natrium/ Sodium Hydroxide
No.	Number
OD	Optical density
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
pH	per hydrogen
RAPD	Random Amplified Polymorphic DNA
REP	Repetitive element sequence-based
Rep-PCR	Repetitive Element PCR
ResAP-PCR	Restricted Arbitrarily Primed PCR
RNase	Ribonuclease
rpm	Revolutions per minutes
s	seconds
S. Typhi	*Salmonella enterica* serovar Typhi
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
Tris	Tris (Hydroxymethyl) methylamine

UV	Ultraviolet
VNTR	Variable-Number Tandem Repeat
w/v	Weight per unit volume