RESTRICTED AP-PCR FINGERPRINTING OF *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM

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ABSTRACT

Salmonella enterica serovar Typhimurium is one of the major serotypes that cause human and animal infections. *Salmonella* infections are capable of producing serious infections that are often foodborne and present as gastroenteritis. Identification of an epidemic strain such as, *Salmonella* Typhimurium is often critical to the success of epidemiological investigations aimed at preventing the spread of infection and eradicating its source.

In this study, 55 *S.* Typhimurium strains including human and animal sources were collected from sporadic cases of salmonellosis during the years 1969 to 2006 in Malaysia and, were characterized using restricted arbitrarily primed polymerase chain reaction (resAP-PCR).

First, confirmation PCR was carried out to confirm the identity of strains as *S.* Typhimurium serotype. The result showed, that 49 strains were confirmed as *S.* Typhimurium (14 strains from humans and 35 from animals) as indicated by expected amplicon with approximate size of 401 bp.

Furthermore, genomic DNA of 49 *S.* Typhimurium strains were extracted followed by digestion of extracted genomic DNA by using *Hae*III restriction enzyme.

A restricted AP-PCR (resAP-PCR) was applied on digested genomics DNA of 49 *S.* Typhimurium strains by using these different primers ResAP-PCR I, ResAP-PCR II and ResAP-PCR III to explore the utility of this method to subtype the strains.

Fingerprinting was scored and comparisons were made using GelCompare II software and clustering was done based on unweighted pair group average method

(UPGMA) Based on 70% similarity 2 major clusters with total of 19 banding profile patterns were generated

In terms of typeability, the *S*. Typhimurium strains were typeable by using resAP-PCR in assigning a defined type to each isolate tested. In terms of reproducibility, resAP-PCR was highly reproducible.

Restricted AP-PCR amplification of *Hae*III digested DNA using ResAP-PCR I, ResAP-PCR II and ResAP-PCR III primers discriminated 49 strains of *S*. Typhimurium into 19 AP profiles. This method had a moderate discrimination index (D = 0.71) and it was not a powerful method in subtyping of *S*. Typhimurium strains.

Cluster A is a major cluster comprising of two subclusters, A_1 and A_2. Subcluster A_1 is contained 24 strains. It is included zoonotic (n=18), one human (n=6) and 58% of them were isolated in year 2005. From 18 zoonotic strains, chicken strains were the majority with n= 9 where 66% of them were isolated in year 2005. Subcluster A_2 contained 6 zoonotic strains (chicken=4, cattle=1, animal=1) and 2 human strains isolated between 1970 until 2006.

Cluster B comprised of two subclusters, B_1 and B_2. Subcluster B_1 consisted of 6 strains, 3 from chicken (isolated in year 2005), 2 from humans (isolated in year 1970), and 1 from zonootic. The AP2 which present in 3 strains (STM 95893/70= human, STM 30822/70= human, STM 3503/05= chicken) was the majority of all profiles. All chicken strains in this subcluster were isolated in year 2005. Subcluster B_2 consisted of 5 strains comprising two AP profiles. AP18 was presented in 3 strains (STM 113254/70= human,

STM 0504/69=human, STM 1204/05= chicken). AP5 was observed in two strains (STM 5553/04=animal and STM 2553/04=cattle)

Overall, restricted AP-PCR analysis was of limited value for subtyping of *S.* Typhimurium isolates from humans and animal sources due to the low discriminatory power. The results revealed that resAP-PCR analysis of *Hae*III digested S. Typhimurium DNA using ResAP-PCR I, ResAP-PCR II and ResAP-PCR III primers demonstrated low polymorphism and low diversity among *S.* Typhimurium isolates.

In conclusion, although restricted AP-PCR method was reproducible, it was moderately discriminative (D=0.71). The use of restriction enzymes to increase polymorphism and discrimination was offset by the additional costs and steps in analysis.

ABSTRAK

Salmonella enterica serovar Typhimurium merupakan serotaip utama yang mengakibatkan jangkitan pada manusia dan haiwan. Jangkitan Salmonella merupakan jangkitan yang serius dan sering disebabkan oleh makanan. Identifikasi strain epidemik S. Typhimurium adalah kritikal dan penyelidikan epidemiologi adalah sukar.

Dalam kajian ini, 55 strain S. Typhimurium termasuk yang berasal daripada manusia dan haiwan, telah dikumpul dari kes-kes salmonellosis dari tahun 1969 hingga tahun 2006. Strain ini telah dikenal-pasti dengan menggunakan restricted arbitrarily primed polymerase chain reaction (resAP-PCR).

Pengenal-pastian dengan menggunakan PCR telah dijalankan. Keputusan menunjukkan bahawa 49 strain adalah S. Typhimurium (14 dari menusia dan 35 dari haiwan) seperti yang ditunjukkan pada amplicon TYPH yang bersaiz 401 bp.

Disamping itu, genomic DNA pada 49 S. Typhimurium diekstrak dan DNA yang berkualiti tinggi dihadam dengan menggunakan enzim HaeIII.

Dalam kajian ini, satu 'restricted AP-PCR' diaplikasi untuk menaip 49 S. Typhimurium dengan menggunakan primer-primer ResAP-PCR I, ResAP-PCR II, dan ResAP-PCR III, untuk mengkaji kebolehannya untuk mengenal-pasti strain.

Pembezaan fingerprinting dijalankan dengan menggunakan GelCompare II dan klusterfikasi dijangka dengan menggunakan 'unweighted pair group average' (UPGMA). Berdasarkan 70% kesamaan, 2 kluster utama telah dihasilkan dengan sejumlah 19 banding profile patterns.

S. Typhimurium dapat dikenal-pasti dengan menggunakan resAP-PCR dengan menetapkan jenis setiap isolate. ResAP-PCR dapat memberi kehasilan yang tinggi.

Restricted AP-PCR yang mengamplifikasikan DNA yang dihadam oleh HaeIII dengan menggunakah primer-primer ResAP-PCR I, ResAP-PCR II, dan ResAP-PCR III dapat mengdiskriminasi 49 strain *Salmonella* Typhimurium kepada 19 profil AP. Keputusan pada dendogram dengan index diskriminasi (D = 0.71) menunjukkan kaedah ini adalah tidak sesuai untuk menaip *S.* Typhimurium memandangkan kaedah ini adalah 'moderately discriminate'.

Kluster A merupakan cluster utama dan mengandungi dua *subcluster*, A1 and A2. Subcluster A1 mengandungi 24 strain, termasuk zoonotic (n=18), satu manusia (n=6) dan 58% strain ini dikumpul dalam tahun 2005. Daripada 18 zoonotic strain, strain daripada ayam merupakan majoriti dengan n= 9 di mana 66% daripadanya telah dikumpul dalam tahun 2005. Subcluster A2 mengandungi 6 zoonotic strain (chicken=4, cattle=1, animal=1) and 2 strain manusia yang dikumpul dari tahun 1970 hingga tahun 2006.

Kluster B mengandungi dua subcluster, B1 and B2. Subcluster B1 mengandungi 6 strains, 3 dari ayam (dikumpul pada tahun 2005), 2 dari manusia (dikumpul pada tahun 1970), dan 1 dari zonootic. AP2 dalam 3 strain (STM 95893/70=manusia, STM 30822/70= manusia, STM 3503/05=ayam) merupakan majoriti dalam semua profil. Semua strain ayam dalam subcluster ini dikumpul dalam tahun 2005. Subcluster B2 mangandungi 5 strain dengan dua AP profil. AP18 terdapat dalam 3 strain (STM 113254/70=manusia, STM 0504/69=manusia, STM 1204/05=ayam). AP5 terdapat dalam 2 strain (STM 5553/04=haiwan and STM 2553/04=lembu).

Secara keseluruhan, analisa restricted AP-PCR tidak seberapa sesuai untuk menaip *S.* Typhimurium daripada manusia dan haiwan disebabkan kuasa diskriminasi yang rendah. Keputusan menunjukkan analisa resAP-PCR dengan DNA *S.* Typhimurium yang dihadam oleh *Hae*III menggunakkan primer-primer ResAP-PCR I, ResAP-PCR II, dan ResAP-PCR III menunjukkan polymorphism yang rendah, dan diversiti yang rendah.

Kesimpulannya, walaupun kaedah restricted AP-PCR mempunyai kehasilan yang tinggi, kaedah ini hanya "moderately discriminative" (D=0.71). Kegunaan enzim restriction untuk meningkatkan polymorphism dan diskriminasi memerlukan kos tambahan dan kaedah analisa.

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ABBREVIATIONS

>	Greater Than
=	Equals to
ºC	Degree Celsius
µl	Microliter
µg	Microgram
µm	Micrometer
%	Percent
AP	Arbitrarily Primed
bp	base pair
D	Discriminatory Index
dH_2O	Distilled Water
ddH_2O	Double Distilled Water
DNA	Deoxyribonucleotide Acid
EDTA	Ethylenediaminetetaacetic
EtBr	Ethidium Bromide
ERIC	Enterobacterial Repetitive Intergenic Consensus
Fig.	Figure
g	Gram
IS	Insertion Sequence
LB	Luria-Bertani
M	Molar
$MgCl_2$	Magnesium Chloride
mM	Milimolar
mg	Miligram
ml	Mililiter

mm	Milimeter
NaCl	Sodium Cloride
No.	Number
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
pmol	Picomole
psi	Pound Per Square Inch
Ref.	Reference
Rep	Repetitive extragenic palindromic
Res.	Restricted
RNase	Ribonuclease
rpm	Revolutions Per Minute
RFLP	Restriction Fragment Length Polymorphism
Spp.	Species
STM	*Salmonella* Typhimurium
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
Tris	Tris (Hydroxymethyl) Methylamine
UPGMA	Unweighted Pair Group Arithmetic Means Methods
UV	Ultraviolet
V	Volt
XLD	Xylose-Lysine-Desoxycholate
5' –Cs	5 Conserved Segment
3' –Cs	3 Conserved Segment