ABSTRACT

Serotyping is the most fundamental approach in epidemiological surveillance and outbreak investigations for *Salmonella.* Many multiplex PCR assays have been reported for serotyping *Salmonella* based on O and H antigens. The aim of this study was to apply and improve the approach in differentiation and serotyping *Salmonella* enterica strains by combination of sequential multiplex PCRs targeting the O, H and Vi antigens of *Salmonella,* and this achieved by working on 126 strains. All the strains were purified on BSA and produced black colonies of *Salmonella*. The strains were confirmed by PCR amplification gene that targets the *hil*A. 122 of 126 strains produced the expected band size of 789bp. A multiplex PCR (MPCR) for serogrouping targets O and Vi antigens was applied on the 122 strains: 28.7% (C2, n=35), 27.1% (B, n=33), 22.9% (D, n=29), 17.2% (E, n=21), 1.64% (C1, n=2), and 1.64% (A, n=2). Eight of the *Salmonella* strains from group D have the Vi antigen. A multiplex PCR for serotyping targets H1 antigens was applied on the tested strains, and were Ha, Hb, and Hd antigens detected in 19 of 122 strains. *S*. Paratyphi A and *S*. Typhi correctly are identified by MPCR for H1 antigens. The percentage of the strains were produced flagella antigens (H1; a, b, and d) was 18.1% while the percentage of non-expressed strains was 81.9%. Therefore, due to the limited information provided by H1 MPCR assay, additional primers for flagella antigens (both H1 and H2) were used. The optimization was carried out on the new primers by adjusting the concentration of buffer, time and denaturation and annealing temperature. The optimized PCR for H serotyping detected the expected amplicons of H1 and H2 antigens and further confirmation was done by DNA sequencing. The overall of multiplex PCRs of O and H

results correctly serotyped 94 of 122 strains (77%). The most frequent serovars encountered were *S.* Weltevrerden, *S.* Enteritidis, *S.* Typhimurium, *S.* Hadar and *S.* Typhi. Application of DNA based serogrouping and serotyping found to be robust, quick methods for differentiation of common *Salmonella enterica.*

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LIST OF ABBREVIATIONS

Abbreviations	Stand for
BSA	Bovine serum albumin
DNA	Deoxyribonucleic Acid.
dNTP	Deoxynucleotide triphosphates
MgCl2	Magnesium Chloride
Taq	Thermus aquaticus
Ml	Microlitre
μM	Micromolar
Mg	Milligram
UMMC	Universiti Malaya Medical Centre
USM	Universiti Sains Malaysia
DMSO	Dimethyl sulfoxide
LB	Luria-Bertani
PCR	Polymerase Chain Reaction.
mPCR	Multiplex polymerase chain reaction
ddH2O	Double-distilled water
TBE	Tris-borate EDTA
Bp	base pair
Ml	Milliliter
mM	Millimolar

U	Unit
G	Gram
IMR	Institute of Medical Research
WHO	World Health Organization
&	And