



A simplified multiplex PCR-based typing method for common *Salmonella enterica* serovars supported by online server-based detection system

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Received August 8, 2015

Background & objectives: A rapid and simple alternative method is needed to replace the laborious, time-consuming *Salmonella* serotyping. The objective of the present study was to improve and simplify a previously reported multiplex polymerase chain reaction (PCR)-based method and to create an online server to enable rapid determination of serovars.

Methods: A method of multiplex PCR-based genome typing (MPGT) was standardized using 59 *Salmonella* isolates of 31 serovars. Several previously reported primers were modified to obtain a more accurate performance. The screen was separated into four different multiplex reactions distinguishable on standard electrophoresis. A blind study was subsequently performed with 81 isolates of 10 serovars most prevalent in India. Whole genome information from 440 *Salmonella* isolates was used to confirm the usefulness of this method and concurrence of *in silico* predictions and PCR results were investigated. A public server (<http://www.mpgt-salmonella.res.in>) was established for data storage and determination of closest previously observed *Salmonella* isolates based on obtained MPGT patterns.

Results: The 16 target genes amplified showed variability in their presence in strains from different serotypes. Hence, identical amplification patterns suggested genetic relatedness of strains and usually identical serological behaviour. The observed absence/presence patterns of genes were converted to an MPGT code. Altogether, 83 different codes were predicted *in silico* based on the whole genome information of 440 strains. Results confirmed that major serovars usually displayed unique MPGT codes.

Interpretation & conclusions: The multiplex PCR assay resulted in specific binary codes for isolates from each of the 31 *Salmonella* serovars tested. The online server allowed the user to compare obtained PCR results with stored previous patterns. Simplicity, speed and cost-effectiveness make this tool useful for quick outbreak management.

Key words Molecular typing - multiplex polymerase chain reaction - public database - *Salmonella* - serovar

Salmonellae are one of the leading causes of community-acquired food-borne bacterial gastroenteritis worldwide. Salmonellae are divided into two species, *Salmonella bongori* and *Salmonella enterica*. The species *S. enterica* is further divided into six subspecies, namely, *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV) and *indica* (VI)¹. Various strains of *S. enterica* subsp. *enterica* are parasitic in humans and warm-blooded animals and are known to be associated with clinical infections².

Of the known >2500 serovars of *Salmonella*, about 1500 belong to *S. enterica* subsp. *enterica*³. Serotyping of *Salmonella* strains is conventionally carried out by identification of surface antigens [lipopolysaccharides (LPS), O-antigens] and flagellar antigens (proteins, H-antigens). Although serotyping using the Kauffman-White scheme remains the standard for serovar determination because of its long-standing and widespread use, it has certain deficiencies. Besides being labour-intensive and expensive, serotyping is also time consuming, often requiring three or more days for a highly trained laboratory technician to produce a result⁴.

Sometimes, atypical expression of surface O or H antigens of an isolate may lead to incomplete or incorrect identification of the serovar. Genomic studies of common clinical serotypes have also revealed a high level of genomic variation amongst isolates of some serovars^{5,6}. It has been suggested that on the basis of genetic relatedness amongst isolates within the same serovar, a 'genovar' (more precisely, 'genomovar') classification may be adopted^{7,8}.

With the increased availability of genomic sequence information, attention has been focused on exploiting this information for finding a molecular strategy of identification of antigenic diversity amongst *Salmonella* strains, either as a complementary technique or as replacement for conventional serotyping. Approaches include polymerase chain reaction (PCR)-based techniques to determine different O and H antigens^{9,10}, ribotyping¹¹, pulsed-field gel electrophoresis (PFGE)¹², multiplex PCR^{11,13}, IS200 analysis¹⁴, random amplification of DNA polymorphisms¹⁵, multilocus sequence typing^{16,17}, matrix-assisted laser desorption ionization-time of flight (MALDI-TOF)-mass spectrometry (MS)¹⁸ and DNA microarray analysis¹⁹. These typing methods have been reviewed by Al-Mogbel *et al.*²⁰.

Based on the genetic differences amongst the serotypes, Leader *et al.*⁴ reported the development of a rapid, high-throughput multiplex PCR-based method that was able to discriminate the majority of common serotypes reported in the United States. The use of this typing method, especially in conjunction with serogrouping or PFGE, allowed for serovar determination of *Salmonella* isolates at a level comparable to that of conventional serotyping, with considerable time and cost savings.

However, as the method involves fluorescent labelling of the products and subsequent detection by capillary electrophoresis, it may not be suitable for use in routine laboratories in developing countries. The present study was undertaken with a view to increase the specificity and sensitivity of this method by modifying some primers and to simplify the protocol by obviating fluorescent labelling and replacing the capillary detection system with conventional gel electrophoresis. The study was further extended by developing a freely accessible online server for storage and expansion of obtained multiplex PCR patterns to facilitate rapid determination of serovars by correlating the patterns with previously deposited patterns.

Material & Methods

A total of 45 *Salmonella* isolates belonging to 22 different serovars (at least two isolates per serovar) were obtained from the strain collection at University of California (UC), Irvine. Additional 14 *S. enterica* subsp. *enterica* isolates of serovars Typhimurium (2), Typhi, Paratyphi A, Virchow, Newport, Schwarzengrund, Enteritidis, Worthington, Gallinarum, Infantis, Idikan, Vridi and Paratyphi B were obtained from the Microbial Type Culture Centre, Chandigarh, and the National Institute of Cholera and Enteric Diseases, Kolkata, India. These strains were used in the code determination phase of the project. For code verification experiments, 81 additional strains (22 Enteritidis, 16 Typhi, 15 Typhimurium, 10 Newport, 8 Weltevreden, 4 Dublin, 2 Litchfield, 2 Gallinarum, 1 Paratyphi B and 1 Virchow) isolated in India from May 2011 to December 2014 from human (28), poultry (23), cattle (18), wild birds (7), pig (2), tiger (1), snake (1) and mouse (1) were tested. The part of the work involving strains (45) from UC, Irvine, including the *in silico* analysis of genomic sequences (440) was done in the department of Microbiology and Molecular Genetics, University of California, Irvine, USA, between June and November 2010. The part

involving Indian isolates (95) including creation and hosting of database was done in College of Veterinary Science, Assam Agricultural University, Guwahati, India, during 2011-2014.

For preliminary standardization of the method, a DNA template from the sequenced strains of *S. enterica* serovar Typhimurium LT2 (NCBI Bioproject #PRJNA57799), *S. enterica* serovar Typhi CT18 (PRJNA57793) and *S. enterica* serovar Enteritidis P125109 (PRJNA31109) were prepared using the Sigma GenElute Bacterial DNA kit (Sigma-Aldrich Corp., St. Louis, MO, USA). These isolates were also tested through colony PCR²¹, where a colony suspension was boiled in a total volume of 75 µl of ×1 TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for 20 min at 100°C and then used directly in the multiplex reaction. DNA of the other strains investigated was prepared using the Sigma GenElute Bacterial DNA kit.

Multiplex PCR: Four multiplex-specific 10x primer master mixes were prepared to contain 4 µM of each primer. All PCRs were carried out in a final volume of 25 µl containing 0.5 µl of *Taq* DNA polymerase (Qiagen Inc., USA), 2.5 µl of the respective 10x primer master mix and 1 µl of template DNA (either genomic DNA or simple boiled culture preparations in TE, pH 8). Thermocycling parameters were 94°C for 3 min; 30 cycles of 94°C for 15 sec, 50°C for 30 sec and 72°C for 25 sec; and 72°C for 5 min. Control reaction mixtures containing no template or genomic DNA from *Salmonella* serovar Typhimurium LT2, serovar Typhi CT18 or serovar Enteritidis PT4 were included in each sample run.

Gel electrophoresis and code determination: The PCR products were separated by gel electrophoresis on a 2 per cent (w/v) agarose gel. The resulting product was manually scored, based on the presence of a PCR product that corresponded to the predicted amplicon size. Control reactions with DNA from *Salmonella* serovars Typhimurium, Typhi or Enteritidis were employed to verify expected amplicon sizes. A multiplex PCR genome typing (MPGT) code was determined for each tested isolate as follows: all amplicons included in each of the multiplexes (M-1 through M-4) were arranged in descending order of their corresponding product size. The internal control, *Salmonella*-specific region STM1608, resulted in a visible 63bp amplicon in every *Salmonella* isolate and was included in every multiplex. This control reaction was not integrated into the code. For the other amplicons, a successful PCR

product was then indicated by 1, a failure to amplify by 0. The resulting code consisted of four blocks of binary numbers, separated by hyphens, corresponding to the four multiplexes used in this identification scheme (M1-M2-M3-M4). Each digit corresponded to a gene in these multiplexes.

Optimization of primer sequences and concentrations: For modification of the PCR regimen employed by Leader *et al*⁴, all original 16 target genes were retained, but the fluorescent-labelled universal probe sequence tag was eliminated from each primer. The sequences of 18 of the 32 original primers employed in that study⁴ were preserved. Performance of the remaining 14 primers (STM1608R, STM0839F, STM0839R, STM1350R, STM4538F, STM0716F, STM0716R, STM3845F, STM3845R, STM4525F, STM4525R, STM3518F, STY0311F and STY0311R) was found to be suboptimal in the present study. These primers were modified, either through shifting of the target sequence by two or three nucleotides or through an entirely new design using Primer-BLAST against the previously reported target gene sequences. The Table lists all primers used in the modified protocol.

Based on their expected product sizes, the primer pairs were divided into four separate multiplex sets (M-1, M-2, M-3 and M-4, Table), allowing easy separation of the amplified products during standard gel electrophoresis. Primers directed towards a universal *Salmonella*-specific region (STM1608) were incorporated in each of the multiplex groups as internal DNA amplification control.

The observed absence/presence patterns of the multiplexed PCR reactions were converted to an MPGT code. The identical amplification patterns suggested relatedness of strains and usually an identical serological behaviour. Subsequently, a more in-depth blind study was performed for the most important and/or common isolates prevalent today (code verification). For this, 40 isolates belonging to five different serovars (Typhimurium, Typhi, Enteritidis, Gallinarum and Dublin) were tested.

In silico determination of multiplex PCR-based genome typing (MPGT) codes for Salmonella genomes: A total of 440 *Salmonella* genome sequences available as NCBI BioProjects (<https://www.ncbi.nlm.nih.gov/bioproject>) were interrogated for determination of their theoretical MPGT codes. These sequences included at least 85 different serovars and isolates from all six

Table. Primers used for determination of *Salmonella enterica* serotypes by multiplex polymerase chain reaction

Accession of target genome	Primer name	Primer sequence (5' - 3')	Amplicon size (bp)	Multiplex group
AE006468	STM1608F	GTCGGCGAGCTTCACC	63	All
	STM1608R	CATCGTAGCAAAGCGCAGTT		
AE006468	STM0839F	AAGGTTTCCCGCTGACCTCT	81	M-3
	STM0839R	AGGTACGCATCCATACGCCT		
AL627273	STY2296F	CTCTGTTGCAAACCAGATGTTA	86	M-1
	STY2296R	GATGGAGACGATAAGTTTACCAGTAT		
AL627273	STY2349F	GCGGAGTTCTACGCAGAGATAA	91	M-2
	STY2349R	CCAGCGAAATGTCACAGTGA		
AE006468	STM1350F	GGAACACATCTTGCCAGGT	108	M-4
	STM1350R	CCGGCACAGAAGGAATGC		
AE006468	STM4538F	AGCGGAATGATGGAAAGCCTA	118	M-3
	STM4538R	CTTCTCCCGTACCAAACGTA		
AE006468	STM0716F	CCGGAAAGAAACCGCTGCTTA	125	M-2
	STM0716R	TTGAGGCGCCGGATATAAAG		
AE006468	STM3845F	GTGTTTGAAGATGATATAGCCAGT	138	M-1
	STM3845R	TCCGCTGAAGCAACAATCAG		
AL627266	STY0345F	GGTATGTCGTTCAAACAGGAAT	152	M-4
	STY0345R	CACTGCCGAGCAGTATGAG		
AL627266	STY0311F	TGGTTGCCACCTATACGTCT	172	M-1
	STY0311R	GTTCACTGGCGTATCCGGTA		
AE006468	STM0171F	GACCCCGGATTTTTTGAGAA	179	M-2
	STM0171R	ACCACGGAGAGACAGTTCAGAT		
AE006468	STM4525F	CTGAGCATCCGCTTCATCGC	239	M-1
	STM4525R	ACACTGACCAGCACCAGG		
AE006468	STM2150F	AGCCTGCATAATCGCAAAGG	198	M-1
	STM2150R	CATCAGCGACACGATAGTGAGA		
AE006468	STM3518F	CGCGGAAGCGGTAGCTA	230	M-4
	STM3518R	CAAGATAGCCACTTTCGGTTG		
AF370716	SdfIF	GCGGATATAAGTACGACCATCATGG	225	M-2
	SdfIR	GCACGCGGCACAGTTAAAA		
AE006468	STM2771F	CCATTGGATGTCCTCACACC	231	M-3
	STM2771R	GGCAATCTTGAAGAATTATCAGG		

S. enterica subspecies. BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was employed to investigate primer hit locations in these genomes, using relaxed similarity parameters (minimum bit score=20 and minimum word size=8) and absence or presence of the desired PCR product was inferred.

Server-based multiplex PCR-based genome typing (MPGT) pattern comparisons: An online database and server supported by a database (<http://www.mpgt-salmonella.res.in>) were developed for quick and simple

comparison of the multiplex PCR pattern obtained from an unknown test strain to previously observed patterns, with the inference of close relationships. The implementation of the database was carried out on the WAMP (Windows, Apache, MySQL, PHP) platform. The user connects to the MySQL-MPGT code database through a web browser.

The database consisted of reference data pertaining to 31 clinically important *Salmonella* serovars, their MPGT codes and the corresponding antigenic

properties. In addition, the database also included predicted MPGT codes of at least 85 *Salmonella* serovars based on *in silico* analysis of 440 *Salmonella* genome sequences available as NCBI BioProjects. After entry of vital strain metadata such as location, host and date of its isolation and associated clinical symptoms, the user simply indicates the status of the targeted amplicons by clicking on the corresponding text boxes against the specific products (Fig. 1). All submissions made by a registered user are stored in the server for future references.

If the user's submitted results do not match any existing MPGT codes in the database, the five closest matches (serovars and their MPGT codes) will be displayed, based on a scoring matrix developed for this purpose. Briefly, the MPGT codes of user inputs and those in the database are split from strings into characters and fetched into arrays. After that, the elements of the users' arrays are matched 1:1 (proceeding from index zero to the last element of the array) with MPGT codes of each of the serovars fetched earlier using arrays. If the values match, the score is set to 1, if not, it is 0. The sum of the scores is calculated. Finally, the percentage of total score with respect to the total length of the MPGT codes is calculated. If the score matches 100 per cent, then the server displays the matched serovar along with five nearest matching serovars; otherwise, it shows the nearest five serovars with their matching percentages. The users are able to view not only the serotype of the closest match but also the associated metadata.

Results

The protocol standardized for the multiplex PCR reactions using one standard strain each of *S.*

Typhimurium (LT2), *S. Typhi* (CT18) and *S. Enteritidis* (PT4) yielded distinct band patterns of the target genes with expected product sizes on agarose gel electrophoresis (Fig. 2).

Overall, 83 different MPGT codes were predicted for the 440 strains. Results confirmed that the major serovars usually resulted in separate and unique MPGT codes, exemplified by the Typhimurium-specific code (11010-0110-111-101), found in 22 Typhimurium isolates, including the monophasic 4,5,12,i,- variant, the Paratyphi A/Sendai code (00101-0110-100-010), found in four isolates and a distinct code for Enteritidis isolates (00001-1100-011-101), detected in 84 isolates. For Typhi, 14 of the 15 isolates resulted in the same distinct MPGT code (01101-0111-000-010), with only Typhi Ty2 displaying a single-digit predicted difference (01001-0111-000-010). Occasionally, closely related strains of different serovars resulted in identical predicted MPGT codes (for example, strains of the serovars Gallinarum and Dublin, code 00001-0100-011-101, Kentucky and Hadar, code 01000-0100-111-001), and some serovars displayed a variety of codes (such as Montevideo, where 41 of the 53 isolates displayed a prominent code 00110-0100-010-001, but the remaining displayed four variations of MPGT codes). Those serovars may be of polyphyletic origin as previously observed²². However, limited utility of the multiplex PCR approach was observed for the distinction of isolates from subspecies other than subspecies I, as many of these failed to result in a reasonable BLAST hit for most of the primers. Most of these interrogated genome sequences were only high-quality drafts consisting of multiple contigs, so the *in silico* MPGT code determination for these genomes was preliminary.

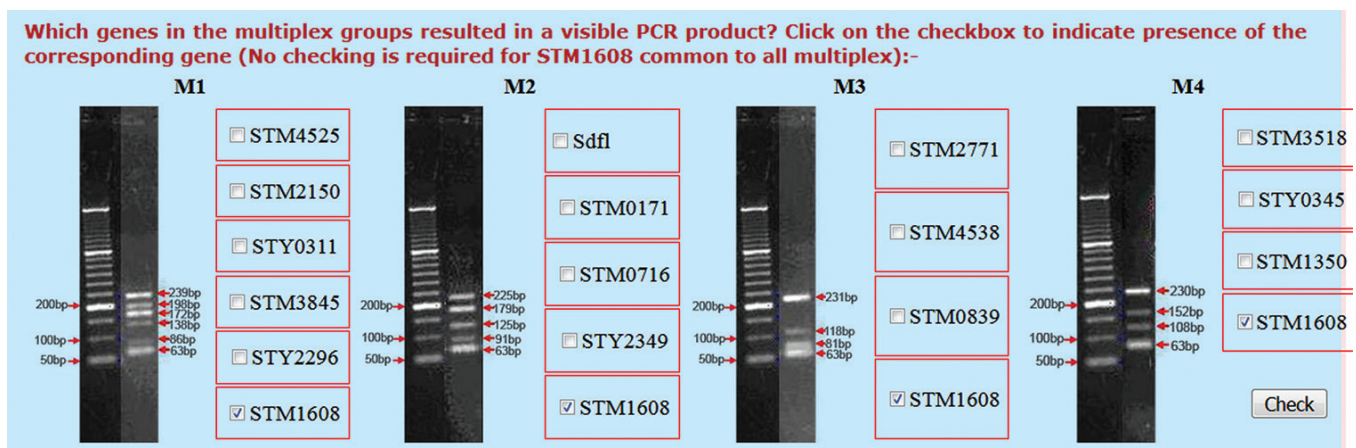


Fig. 1. Screenshot of the data entry window in the multiplex PCR-based genome typing code server.

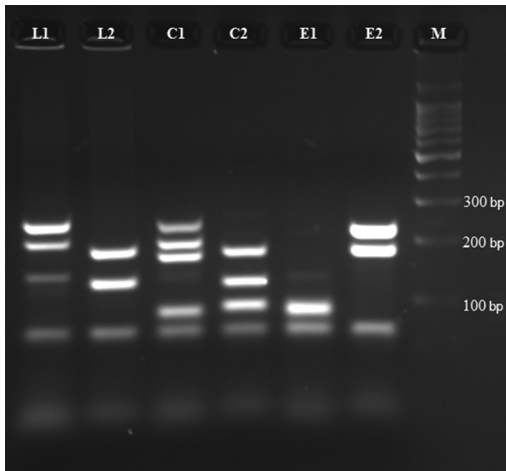


Fig. 2. Amplicons of different sizes obtained by multiplex polymerase chain reaction. L1 & L2, Multiplex (M) 1 and M2 for *S. Typhimurium* (LT2 strain); C1 & C2, M1 and M2 for *S. Typhi* (CT18 strain); E1 & E2, M1 and M2 for *S. Enteritidis* (PT4 strain). M: 100 bp DNA ladder.

PCR-derived MPGT codes for common clinical human and animal Salmonella serovars: Initially, 63 isolates were used to determine wet (as opposed to *in silico*) MPGT codes for 31 *Salmonella* serovars including serovars Typhimurium, Typhi and Enteritidis. For 16 of the 31 serovars tested, a unique MPGT code was produced. Isolates of *Salmonella* serovars Dublin, Gallinarum var. Pullorum, Heidelberg, Kentucky, Paratyphi A, Paratyphi C, Typhi and Weltevreden each produced two MPGT codes. As a notable difference to the *in silico* analysis, Typhimurium isolates also produced two codes, differing by the status of STM3518. Duplicate codes were also obtained for *Salmonella bongori* and *S. enterica* subsp. II, IIIa, IIIb, IV and VI. All serovars screened in the panel resulted in distinct MPGT code identifiers.

In the blind study, 77 (95.06%) of the 81 tested strains resulted in the expected MPGT code identifiers, suggesting a relatively high reproducibility and specificity of the multiplex PCR approach. For *Salmonella* Typhimurium, 13 of 15 isolates resulted in an MPGT code that corresponded to the code produced by Typhimurium isolates during code determination experiments. Amongst these 13 isolates, seven showed the specific *in silico*-predicted MPGT code (11010-0110-111-101) and the remaining six displayed the alternative code (11010-0110-111-001), which was deficient of the gene *STM3518*. However, two of the 15 tested strains that had been stocked as Typhimurium resulted in an MPGT code (00001-1100-011-101)

produced by Enteritidis strains during code determination (a code that differs from expected Typhimurium codes by at least 8 out of 16 sites), indicating a high likelihood of mistyping or misrecording of these strains. Twenty one of 22 tested isolates of *Salmonella* Enteritidis resulted in MPGT codes indicative of serovar Enteritidis (00001-1100-011-101). The remaining isolate tested displayed a MPGT code indicative of serovar Gallinarum in our code determination tests (00000-0110-101-001), suggesting a typing or recording error. All 16 isolates of the human host-specific serovar *S. Typhi* were correctly identified by the multiplex PCR method. Nine of these showed the typical code (11101-0111-000-010), whereas seven displayed a code devoid of gene *STM4525* (01101-0111-000-010), the prevalent Typhi code identified in the *in silico* analysis. All eight isolates of *S. Weltevreden* were correctly identified by the method, of which four each showed the alternative codes (00110-0110-110-001 and 10110-0111-110-001). Three of the four tested isolates of *Salmonella* Dublin resulted in the expected MPGT code (00001-0100-011-101), and the remaining one resulted in an MPGT code identical to a code previously obtained from a Typhimurium isolate (11010-0110-111-101, a difference in 6 sites), underlining utility of the MPGT screen to identify strains that had been misrecorded. Both tested isolates of *Salmonella* Gallinarum resulted in their expected MPGT code (00000-0110-101-001). Both isolates of *S. Litchfield* (11001-0110-111-001) and the single isolate each of *S. Paratyphi* B (11000-0110-110-001) and *S. Virchow* (01000-0110-101-001) tested in blind study showed codes typical of the serovars as determined earlier by code determination with standard strains.

Comparison of PCR results with in silico predictions: For 13 of the 31 tested serotypes investigated in the code determination experiments, obtained patterns concurred entirely with codes predicted by the *in silico* analysis for isolates of the same serotype. This lower than expected concordance prompted us to compare *in silico* and laboratory patterns obtained for the 28 strains where both analyses had been performed. Overall, *in silico* analysis correctly predicted the status of 381/420 amplification products (90.7%). All but four of the discrepancies were cases where the *in silico* analysis failed to predict an obtained PCR product, suggesting either PCR product generation based on weak sequence similarities that cannot be picked up by a simple BLAST search or, alternatively, unexpected product generation due to primer crosstalk.

Discussion

Although conventional serotyping offers a reliable method for differentiating *Salmonella* strains, it is a time-consuming process. Among other methods, PFGE is highly discriminative for *Salmonella*, but it is expensive and time consuming. Further, its standardization, analysis and comparison of restriction profiles require effort²⁰. Ribotyping is a labour-intensive and time-consuming technique, analysis may take in some cases 4-5 days to complete²³. IS200 profiling has been found to have low discriminatory power²⁴. The PCR-based methods such as RAPD lack the reproducibility between laboratories²⁵. Similarly, the results of ERIC-PCR are usually less discriminatory²⁶. MLST does not provide the fine resolution needed for outbreak analysis and short-term epidemiology²⁰. Although Matrix-assisted laser desorption/ionization Time-of-flight Mass Spectrometry (MALDI-TOF-MS) can be a rapid method for typing, laborious control of the sample preparation and optimization of testing parameters are crucial for strain typing with this method, which is not practical in clinical laboratories²⁷.

The combination of the four multiplex PCRs reported here resulted in specific binary codes for isolates from every one of 31 serovars tested in code determination experiments including isolates from the most common and/or most severe serovars (Typhimurium, Enteritidis, Typhi and Paratyphi A). For 16 of the 31 serovars tested, a single MPGT code was produced for each serovar.

The code verification experiments correctly identified the closest relative within the expected serovar in >95 per cent of the examined *Salmonella* strains. This imperfect concordance level may, in part, stem from possible cross-reaction of the primers in the multiplex combinations with each other or certain target DNA sequences. This was supported by the fact that the *in silico* analysis correlated not entirely with the obtained laboratory PCR results and failed to predict a few observed amplified products. However, some of the inconsistencies may also be caused by incorrect conventional serotyping based on human error or the quality of the antiserum lots²⁸ and by incorrect recording of stocked isolates. Finally, it cannot be assumed that strains will result in patterns that have been observed for other isolates of the same serovar. It is known that serotype is not always a measure of genetic relatedness²⁹, and therefore, a DNA-based method like this multiplex PCR approach may initially miscategorize observed amplification

patterns. However, as the server is constantly enriched by more observations, the last type of discrepancy will become less relevant.

For most of the strains tested in the present study, the gene profiles corresponded to those previously reported⁴ for all 16 loci. However, some discrepancies were observed. Unlike the results reported by Leader *et al*⁴, gene *STM4525* was detected in our multiplex PCR test for two isolates of Typhi and all three Paratyphi A isolates tested. Our primers for this target gene differed from those originally reported in that study⁴. The *in silico* analysis suggested this gene to be absent from Typhi and Paratyphi A isolates, and amplification of this product by PCR may, therefore, constitute a template-specific artefact. Similarly, *STY2349* was inexplicably but repeatedly amplified from single isolates of Choleraesuis, Kentucky and Virchow, and *STM0839* was detected in one Heidelberg strain.

The major advantages of using the present multiplex PCR to determine *Salmonella* relatedness were its speed, simplicity and relative accuracy. An isolate could be typed within 1.5 to two hours with a reasonable level of accuracy, a clear advantage over the conventional serotyping method. Although conventional serotyping is considered the gold standard for *Salmonella* identification, it occasionally demonstrates inconsistent results³⁰.

An additional benefit of the multiplex PCR method is the possibility to determine a molecular profile for those strains that cannot be serotyped (*i.e.*, those that do not express antigen or have an LPS defect rendering them untypable). This occurs for 5-8 per cent of all strains routinely tested in laboratories¹³. Compared to other methods such as PFGE, ribotyping and MALDI-TOF-MS, the present method is easier to perform, less expensive and less time consuming. However, this method needs further validation against type cultures of other common clinical serovars and a large number of field isolates. Users can submit an obtained novel code to the MPGT database either as a new serovar (known or unknown) or as an alternative code for an existing serovar. Through user input, the database is expected to expand over time with continuous incorporation of data pertaining to more and more strains and serovars.

In conclusion, the present multiplex-PCR-based method supported by the freely-accessible online server provided a simple, rapid and reasonably accurate alternative to conventional serotyping for determination

of serovars in clinical isolates of *Salmonella*. As more and more MPGT codes get incorporated into the database, accuracy of its closest relative predictions will improve, and its utility vastly expanded. The simplicity of the method allowed this crude molecular typing of common clinical *Salmonella* isolates to be performed in any laboratory without much sophistication. This method could be useful for quick outbreak management.

Acknowledgment

The authors acknowledge the Department of Biotechnology, Govt. of India, for providing necessary financial support in the form of DBT Overseas Associateship provided to the first author and subsequent funding in the form of DBT-NER Twinning Project (BT/375/NE/TBP/2012) to carry out the study.

Conflicts of Interest: None.

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