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Typing of Indian S.Typhi Isolates with Special Reference to Pulsotypes

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Abstract: ***Six hundred sixty-four isolates from 23 different centres of North, central and south zones of India were typed by Pulsed Field Gel Electrophoresis (PFGE). Eighty-nine different profiles were delineated which were divided into 16 groups. X-15 was the commonest PFGE profile. The typeability of PFGE and phage typing was 100% and 76.4% whereas the discriminatory power was 0.91 and 0.25 respectively. The dice coefficient varied between 0.64-1. E1 was the predominant phage type. No significant association was found between pulsotypes and phage types of the S.Typhi isolates. X38 pulsotype was associated with ciprofloxacin resistant S.Typhi and X69pulsotype was associated with cefotaxime resistant S.Typhi isolates.***

Keywords: PFGE, S.Typhi, Typing

1. Introduction

Enteric fever is major disease in India and is an important cause of morbidity and mortality (1, 2). Outbreaks especially of MDR S.Typhi have been reported from various parts of India. An important public health measures in controlling these outbreaks is to type these isolates. Till very recently phage typing was the common typing system for typing of these isolates. The outbreaks of MDR S.Typhi in 1989, which peaked in 1992resulting in predominance of E1 phage types strains in India (3, 4). This necessitated the introduction of a genotyping system for the isolates. In the current study, PFGE has been introduced and standardised in the laboratory and the typing techniques has been analysed.

2. Methodology

The present study was carried out at National Salmonella Phage Typing Centre (NSPTC) in the department of Microbiology, Lady Hardinge Medical College, New Delhi from the isolates collected during Jan 2008- Dec 2011. Six hundred and sixty-four strains were selected from over 1323 strains by stratified random clustering method. The list of collaboration centres in presented in table 1.

Table 1: Table List of Collaboration Centres

North Zone	Central Zone	South Zone
1. Lady Hardinge Medical College (LHMC), Delhi 2. Central Research Institute (CRI), Kasauli 3. University college of Medical Sciences (UCMS), New Delhi 4. Batra Hospital, New Delhi 5. Christian Medical College (CMC) Ludhiana, Punjab 6. Dayanand Medical College (DMC), Ludhiana, Punjab 7. Sardar Patel Medical (SPM) College, Bikaner, Rajasthan	1. Kasturba Medical College (KMC), Mumbai 2. Mahatma Gandhi Institute of Medical Sciences (MGIMS) Sevagram, Maharashtra 3. Government Medical College, Nagpur 4. Grant Medical College (GMC), Mumbai 5. Armed Forces Medical College (AFMC), Pune 6. King Edward Memorial (KEM) Hospital, Pune 7. Goa Medical College, Goa	1. Pondicherry Institute of Medical Sciences, Pondicherry 2. Christian Medical College (CMC), Vellore 3. King Institute of Preventive Medicine, Chennai 4. Madurai Medical (MM) College, Madurai 5. Nizam's Institute of Medical Sciences, Andhra Pradesh 6. Sundaram Medical Foundation, Chennai 7. Bose Clinical Lab, Madurai 8. Government Medical College, Thrissur, Kerala 9. Indira Gandhi Institute of Child Health Hospital (IGICH), Bangalore

These isolates were subjected to antimicrobial susceptibility testing using CLSI (2013) guidelines (6).

The technique of bio-typing was performed on the basis of fermentation reaction with xylose and arabinose, using Kristensen technique (7).

Phage typing was done using Andersons Method (8).The pulsed-field gel electrophoresis was performed using PulseNet protocol CDC, Atlanta (9). Gel analysis was performed by Visual Grouping Method by the criteria described by Tenover (10).

The clusters analysis was done by Bionumerics 7.1 software (Applied Maths, Belgium). The Dice coefficient was calculated at 1.5% optimization and 1.5 % tolerance rate. The dendrogram was made by the unweighted pair-group method using arithmetic averages (UPGMA) (10).

The interpretation of banding patterns was done following guidelines suggested by Tenover et al (10). Isolates with identical restriction profiles were assigned the same type and identified with a capital letter X followed by numeral. Isolates that differed from main types by one to three band shifts consistent with a limited number of genetic events

were assigned subtypes. Isolates with more than three such differences were different types (10).

3. Results

In the present study, highest resistance was observed to Ampicillin amongst the first line drugs (11.95 %). The resistance in macrolide category namely, Azithromycin was observed from south Indian zone to be very low rate (0.15%). Resistance to Nalidixic Acid was very high (97.8%). In Ciprofloxacin, most of the strains came in intermediate susceptible category (94.3%). The maximum strains fall in MIC (Ciprofloxacinranges between 0.38-0.5 µg/ml (Figure 1).

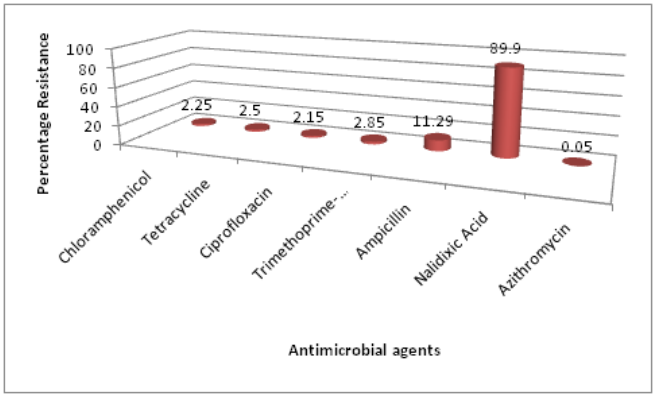

Figure 1: Antimicrobial Resistance Distribution of Indian S.Typhi isolates in India from time period between 2008-2011

Biotyping: More than ninety seven percent of the isolates belonged to Biotype I (660). The prevalence of biotype 1 in North, South and Central zones of India varied between 94.02% to 99.5%. Furthermore 14 biotypes belonged to biotype II and none to Biotype III.

Phage Typing: The prevalence of Phage types of S.Typhi in India in the three zones of India is depicted in Figure 2. Phage type E1 was found to be commonest phage type in India. The prevalence of Phage type E1 varied from 54.4% in North zone to 70.7% in South Zone. Phage types M1and K were limited only to the central and south zones.

Phage type J1, D2, D8 and E7 were limited to north zone and phage type D1, 46, 36 and E4 were South zone. Phage type 40 was isolated only from central zone. The maximum diversity of phage types was seen in south zone whereas the least diversity was observed in central zone ($p < 0.05$).

The isolation rate of phage Type E1 in south zone was significantly higher than the North Zone ($p = 0.0001$). The isolation of UVS in south was significantly higher than in the North zone (p values = 0.049).

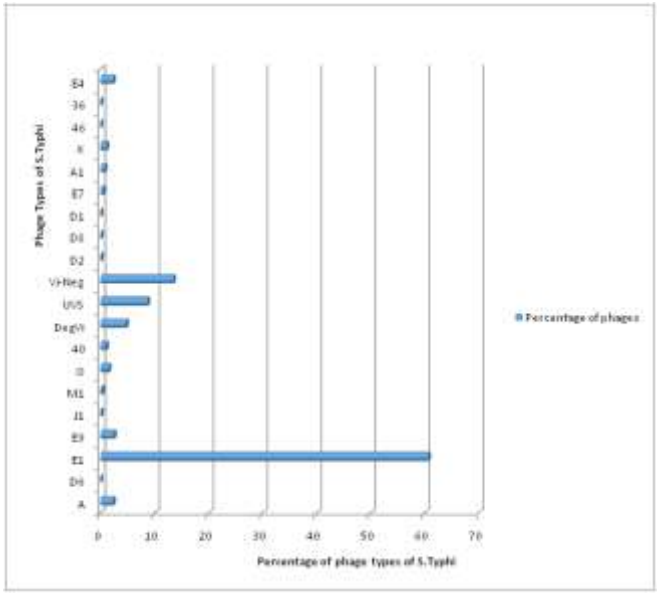

Figure 2: Distribution of phage types of S.Typhi

The PFGE analysis of the isolates was performed using PulseNet protocol (CDC, Atlanta). The PFGE analysis showed bands number varying between 9-17 with sizes in range of 22Kb to 650Kb in the various isolates (Figure 3).

The following pattern of coding of strain was utilised to the strains analysed in the current study.

Geographical Zone	Year	Number of strains	Code
North/ Central/ South	2008/2009/2010/2011	1	N/8/1

For example: In the code N/8/1, N refers to the zone, 8 refers to the test year (last number) and 1 to the strain number

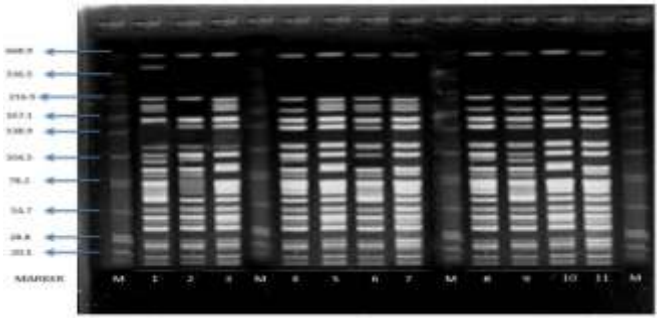

Figure 3: PFGE band patterns of 11 representative S. Typhi strains

1-1/North /2008	**6-6/North/2008**
2-2/North/2008	**7-7/North/2008**
3-3/North/2008	**8-8/North/2008**
4-4/North/2008	**9-9/North/2008**
5-5/North/2008	**10-10/North/2008**
M: Marker fragment length in Kb	**11-12/North/2008**

The dice coefficient varied between 0.64-1 (Figure 4, Table 2). The typeability of PFGE and phage typing was 100% and 76.4% whereas the discriminatory power was 0.91 and 0.25 respectively (Table 3).

The clinical source of isolate was blood in 98.9% cases followed by urine and others. The PFGE profiles did not vary with the clinical source of isolate between 2008-11.The 660 S.Typhi isolate belong to Biotype I only. The PFGE profile did not vary significantly with clinical source of isolate.

The PFGE profiles X7, X15, X27, X34, X56, X67 were commonest PFGE profiles among strains studied in present study PFGE profiles X1, X3, X15, X45, X24, X28, X31, X59, X87, X4, X68, X69, X77, X23, X34, X32, X8, X63, X64, X66, X61, X50, X47, X27, X67, X44, X22, X82 were found in MDR S.Typhi (resistant to Chloramphenicol, Ampicillin and Sulfamethoxazole) strains in India. The highest number of MDR S.Typhi belonged to X15 PFGE type. The PFGE profile X38 belonged to ciprofloxacin resistant S.Typhi and PFGE profile X69 belongs to Cefotaxime resistant S.Typhi in this study.

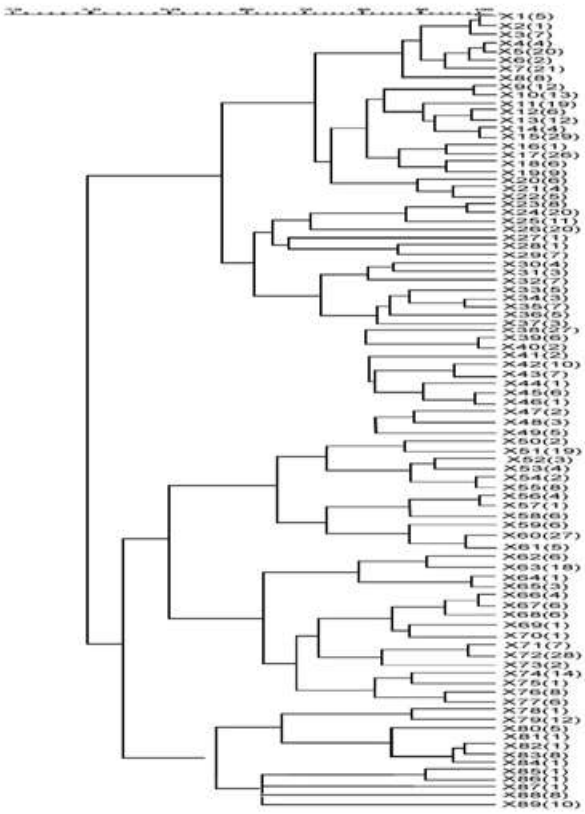

Figure 4: Dendrogram representing relationships amongst different PFGE profiles of S.Typhi isolated during the study period

Table 2: Dice coefficient range of sixteen clusters

Clustering groups of PFGE	Dice coefficient range
1	0.88-1
2	0.82-1
3	0.79-1
4	0.77-0.95
5	0.76-1
6	0.81-1
7	0.79-1
8	0.81-1
9	0.8-1
10	0.88-0.99
11	0.80-1
12	0.76-1
13	0.74-1
14	0.72-1
15	0.70-1
16	0.64-1

Table3: Comparison of Diversity distribution (Discriminatory Index) and typeability of S.Typhi in India by phage typing and PFGE techniques from time period between Jan 2008-Dec 2011

Geographical distribution	North zone	South Zone	Central Zone
PFGE (DI)	0.93	0.97	0.92
Phage Typing (DI)	0.19	0.25	0.9
PFGE (Typeability)	100%	100%	100%
PhageTyping (Typeability)	71.2%	72.1%	78.3%

Statistically there were no significant association between phage types and pulsotypes of S.Typhi ($p > 0.05$).

4. Discussions

The comparison of PFGE Types with the phage types in the Indian isolates did not reveal a significant finding. One of the reasons of this scenario was the predominance of phage type E1, which contributed around 60% and secondly approximately 20% of strains isolate lacked Vi-II antigen in our studies. Similar, observations were found in the earlier studies from Papua, New Guinea, with no association of phage type with pulsotypes (11). The relationship between phage types and PFGE has not been studied in any other Indian study (12-14).

Our study observed X1, X15, X20, X21, X23, X24, X27, X28, X3, X32, X31, X32, X33, X44, X50, X56, X63, X87 pulsotypes in MDR S.Typhi category. Similar findings were found from South India and Pakistan [11, 14].

In our study no correlation was found between PFGE and Nalidixic Acid resistant S.Typhi isolates. However, Nalidixic acid resistance was the most common phenotype and the Nalidixic acid resistance S. Typhi isolate formed one major pulsotype in study from Kolkata isolates (12). However, several other pulsotypes were observed in Vietnam study (14). Le *et al*., suggested that due to increased therapeutic use of fluroquinolones, small scale genetic changes occurred among bacterial generations resulting in formation of diverse PFGE types among Nalidixic acid resistant S. Typhi clones (14).

In our study ciprofloxacin resistant isolates belonged to a single pulsotype X38. Similar observations were found in study from Italy where two CIP-resistant strains isolated from the individuals who visited India exhibited the same PFGE pattern (18). A study from USA on ciprofloxacin resistant isolates of S.Typhi observed indistinguishable PFGE patterns with XbaI (16).

In present study, evinced that multiple MDR clones were found to be co-existing in Indian Subcontinent (12, 15). In concordance with present study another study on strains of

five different countries found different PFGE patterns isolated from the MDR S.Typhi strains from countries of Pakistan and Malaysia (16). Greater number of fragments in PFGE study of MDR S.Typhi was observed from Pakistan as an outcome of insertion of plasmids carrying antibiotic resistance genes in these isolates (17).

The limitation of PFGE is that it is time consuming nature, inability to distinguish between all unrelated isolates, DNA restriction patterns can vary slightly between technicians and cannot optimize separation in every part of the gel at the same time. The bands of same size may not come from the same part of the chromosome change in one restriction site can result in more than one band change, Relatedness" should be used as a guide, not as a true and phylogenetic measure.

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