Phage typing, biotyping & antimicrobial resistance profile of *Salmonella enterica* serotype Typhi from Kolkata


National Institute of Cholera & Enteric Diseases (ICMR), Kolkata, India

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**Background & objectives:** Kolkata and its suburbs in eastern India are known to be endemic for typhoid fever. The objective of this study was to determine phage types, biotypes and antimicrobial resistance patterns of *Salmonella enterica* serotype Typhi isolated during the period 2003-2005 from a prospective surveillance for typhoid fever in two urban slums in Kolkata.

**Methods:** A total of 195 *Salmonella enterica* serotype Typhi isolated from blood cultures were phage typed, biotyped and tested for their antimicrobial susceptibility profile.

**Results:** Phage type E1 was the most common (60.3%) followed by phage type A among five phage types identified. Biotype I (95%) was predominant, 28 isolates were multidrug resistant (MDR) and most of the MDR strains belonged to phage type E1 and biotype I.

**Interpretation & conclusion:** A single phage type and biotype were prevalent among the *Salmonella enterica* serotype Typhi isolates studied from Kolkata, India.

**Key words** Biotypes - phage types - *Salmonella enterica* serotype Typhi

Typhoid fever, caused by *Salmonella enterica* serotype Typhi (S. Typhi) is a major public health problem, particularly in developing countries. Typhoid fever caused 21,650,974 illnesses and 216,510 deaths annually¹. Multidrug-resistant (MDR) S. Typhi have been reported from different parts of India including Kolkata²⁻⁸ and created a significant therapeutic problem. There is wide distribution of drug resistant S. Typhi isolates of different biotypes and phage types throughout the country.

The present study was undertaken to document the biotype, phage type and antibiotic resistance patterns prevailed in recently isolated S. Typhi from eastern region of Kolkata, West Bengal.
Material & Methods

A total of 195 S. Typhi isolates were collected during the period 2003-2005 from a prospective surveillance for typhoid fever in two urban slums in eastern Kolkata. A total of 56,949 individuals were enumerated and enrolled as study subjects for fever surveillance. For each consenting patient from the study population, patients with fever for 3 days or more were included in this study and blood samples were collected and used to inoculate BACTEC Plus Aerobic culture vials (Becton Dickinson, Franklin Lakes, NJ, USA). The BACTEC were incubated at 37°C for 7 days and were visually checked for growth everyday. Bottles were subcultured on MacConkey agar on days 1, 2, 4 and 7 or when turbidity was detected. Suspected non-lactose-fermenting colonies were characterized according to the conventional procedure. There was no dropout as blood was collected during the first visit.

The antimicrobial susceptibility was determined following Kirby and Bauer disk diffusion method in accord with the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) using commercially available antimicrobial discs (Difco, Detroit, USA). Minimum inhibitory concentrations (MIC) of the antimicrobials were tested by “E test” following manufacturers instructions (AB BIODISK, Solna, Sweden).

Escherichai coli ATCC 25922 was used as control strain for antimicrobial susceptibility test.

Phage typing and biotyping were done at the National Salmonella Phage Typing Centre, Department of Microbiology, Lady Hardinge Medical College, New Delhi.

Results & Discussion

Antibiogram revealed that none of the isolates were resistant to amikacin, aztreonam, amoxicillin/clavulanic acid and cephalosporin group of antibiotics (Table I). Sixty six isolates (33.9%) were sensitive to all the antibiotics tested. Chloramphenicol resistance was observed in 32 (16.4%) isolates. Isolates were also found resistant to ampicillin (14.4%), co-trimoxazole (16.9%) and tetracycline (5.6%). Twenty eight isolates (14.4%) were MDR, i.e., resistant against three antibiotics, chloramphenicol, ampicillin and co-trimoxazole. Our findings were similar to those of Das et al. A much higher percentage of MDR (92.3%) S. Typhi was reported earlier from Kolkata. During 1990 to 1992, when Kolkata and its suburbs experienced an epidemic of MDR typhoid fever, all isolates of S. Typhi were uniformly (100%) resistant to chloramphenicol, ampicillin, co-trimoxazole and tetracycline, however, during the subsequent period (1993 to 1997), 30-35 per cent isolates regained susceptibility to these drugs. These changing pattern of resistance continued till 2000. During that period, the bacterium slowly regained susceptibility to these drugs due to the less use and recently Dutta et al. reported a remarkable reversal in the resistance pattern of S. Typhi in Kolkata.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Sensitive (%)</th>
<th>Intermediate (%)</th>
<th>Resistant (%)</th>
</tr>
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<tbody>
<tr>
<td>Amikacin</td>
<td>195 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>195 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>167 (85.6)</td>
<td>0 (0)</td>
<td>28 (14.4)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>195 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>195 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>163 (83.6)</td>
<td>0 (0)</td>
<td>32 (16.4)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>185 (94.9)</td>
<td>8 (4.1)</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>162 (83.1)</td>
<td>0 (0)</td>
<td>33 (16.9)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>72 (36.9)</td>
<td>3 (1.5)</td>
<td>120 (61.5)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>193 (99.0)</td>
<td>0 (0)</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>184 (94.4)</td>
<td>0 (0)</td>
<td>11 (5.6)</td>
</tr>
</tbody>
</table>
Detection of nalidixic acid resistance as a predictor for decreased fluoroquinolone susceptibility in salmonellae has been reported\textsuperscript{15,16}. However, the predictive efficacy of nalidixic acid resistance as a marker of fluoroquinolone resistance is a matter of concern\textsuperscript{17}. In the present study, of the 195 \textit{S. Typhi} isolates tested, 120 (61.5\%) were nalidixic acid resistant (Table I). Of these, 110 were sensitive to ciprofloxacin, 2 were resistant and 8 were intermediately susceptible to ciprofloxacin according to the zone size criteria of manufacturer. Studies from north India also reported the isolation of \textit{S. Typhi} showing high-level ciprofloxacin resistance\textsuperscript{18}. In this study, the two resistant isolates had MIC of 16 \textit{mg/ml} against ciprofloxacin, similar to that reported by Renuka \textit{et al}\textsuperscript{19}. However, the eight isolates had MIC range of 0.125-0.38 \textit{mg/ml} against ciprofloxacin which is in accordance with the report from south India\textsuperscript{20}.

A subset of 116 isolates was subjected to phage typing. The most prevalent phage type was E1 (60.3\%) followed by A (8.6\%) (Table II). A substantial increase in the incidence of phage type E1 was observed from 1992 onwards in Kolkata\textsuperscript{21}. In Ludhiana, phage type E1 was most predominant\textsuperscript{22}. In 1990, O was the predominant phage type in Kolkata\textsuperscript{21}, however, this phage type could not be detected from 1994 onwards till date. Recently from New Delhi and Pondicherry, reports of a very low number of phage type O \textit{S. Typhi} isolates have been published\textsuperscript{23,24}. In the present study, the presence of phage type D1, E9 and 40 were negligible (1.7\% each). Our report contradicts the findings of a study from Mumbai where phage type A was found in highest proportion (45.95\%), followed by phage type E1 (15.32\%)\textsuperscript{25}. Biotyping revealed majority of the isolates (95\%), belonging to group I and remaining 5 per cent to group II. This finding was similar to earlier reports from Kolkata, Nagpur and Manipal\textsuperscript{21,24}. However, Kumar \textit{et al}\textsuperscript{22} reported a higher incidence of biotype II from Ludhiana. Of the 26 MDR isolates subjected to phage typing, 21 belonged to phage type E1 and all belonged to biotype I. The small number of isolates belonging to phage types 40, E9 and D1 were found sensitive to all the antibiotics tested except nalidixic acid, which resembles the report of Pillai \textit{et al}\textsuperscript{25}. The findings of most of the MDR isolates belonging to the phage type E1 and biotype I corroborate with the reports from other parts of this country\textsuperscript{2,4,21,22}.

So, it could be concluded that, a single phage type and biotype were prevalent among the \textit{S. Typhi} isolates, which have been isolated recently from Kolkata, India.

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### References


Reprint requests: Dr S.K. Niyogi, Deputy Director, National Institute of Cholera & Enteric Diseases
P-33, CIT Road, Scheme XM, Beliaghata, Kolkata 700010, India
e-mail: niyogisk@hotmail.com