

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

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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 Kolkata⁹. A total of 56,949 individuals were enumerated and enrolled as study subjects for fever surveillance⁹. 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 detected⁹. Suspected non-lactose-fermenting colonies were characterized according to the conventional procedure¹⁰. 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)¹¹. 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*¹². A much higher percentage of MDR (92.3%) *S. Typhi* was reported earlier from Kolkata¹³. 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 2000¹⁴. During that period, the bacterium slowly regained susceptibility to these drugs due to the less use and recently Dutta *et al*⁸ reported a remarkable reversal in the resistance pattern of *S. Typhi* in Kolkata.

Table I. Antimicrobial susceptibility pattern of *Salmonella enterica* serotype Typhi during 2003-2005 in Kolkata

Antimicrobial agents	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	195 (100)	0 (0)	0 (0)
Amoxicillin/clavulanic acid	195 (100)	0 (0)	0 (0)
Ampicillin	167 (85.6)	0 (0)	28 (14.4)
Aztreonam	195 (100)	0 (0)	0 (0)
Ceftriaxone	195 (100)	0 (0)	0 (0)
Chloramphenicol	163 (83.6)	0 (0)	32 (16.4)
Ciprofloxacin	185 (94.9)	8 (4.1)	2 (1.0)
Co-trimoxazole	162 (83.1)	0 (0)	33 (16.9)
Nalidixic acid	72 (36.9)	3 (1.5)	120 (61.5)
Ofloxacin	193 (99.0)	0 (0)	2 (1.0)
Tetracycline	184 (94.4)	0 (0)	11 (5.6)

Table II. Distribution of different phage types of *Salmonella enterica* serotype Typhi (n=116) during 2003-2005 in Kolkata

Phage type							Biotype				
Typable n=86 (74%)							Untypable n=30 (26%)			I	II
O	E1	A	C9	D1	E9	40	Vi negative	Degraded Vi	UVS		
-	70 (60.3)	10 (8.6)	-	2 (1.7)	2 (1.7)	2 (1.7)	11 (9.5)	10 (8.6)	9 (7.8)	110 (95)	6 (5)

UVS, untypeable Vi-strains
Values in parentheses indicate percentages

Detection of nalidixic acid resistance as a predictor for decreased fluoroquinolone susceptibility in salmonellae has been reported^{15,16}. However, the predictive efficacy of nalidixic acid resistance as a marker of fluoroquinolone resistance is a matter of concern¹⁷. In the present study, of the 195 *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 *S. Typhi* showing high-level ciprofloxacin resistance¹⁸. In this study, the two resistant isolates had MIC of 16 µg/ml against ciprofloxacin, similar to that reported by Renuka *et al*¹⁹. However, the eight isolates had MIC range of 0.125-0.38 µg/ml against ciprofloxacin which is in accordance with the report from south India²⁰.

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 Kolkata²¹. In Ludhiana, phage type E1 was most predominant²². In 1990, O was the predominant phage type in Kolkata²¹, 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 *S. Typhi* isolates have been published^{2,3}. 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%)²³. 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^{4,21,24}. However, Kumar *et al*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 *et al*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^{2-4,21,22}.

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

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