

Isolation of *Salmonella enterica* Serotypes from Children with Diarrhoea in Calcutta, India

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ABSTRACT

The prevalence of *Salmonella enterica* serotypes, antimicrobial susceptibility, and phage typing of seroverters were studied. Clinical presentations of the infected cases were also examined. The study was carried out during August 1993-September 1996 in and around Calcutta, India. In total, 1,025 faecal samples from hospitalized diarrhoeal children were screened for enteropathogens. Four *S. enterica* serotypes were identified in 157 (15.3%) cases as a single pathogen. *S. enterica* serotype Typhimurium was detected in 110 (70%) cases. *S. Seftenberg*, *S. Infantis*, and *S. Virchow* were detected in 28 (17.8%), 14 (8.9%), and 5 (3.2%) cases respectively. *S. Typhimurium* was isolated from 11 (3.2%) non-diarrhoeal control children. All of these children had acute watery diarrhoea, and 5% of them had severe dehydration, 40% had some dehydration, and 55% had no dehydration. Vomiting, fever, and diffused pain in abdomen were the associated presentations of these children. Most (95%) of them recovered with oral rehydration therapy only and without any antibiotics. In-vitro susceptibility testing showed that 120 of the 121 *S. Typhimurium* strains isolated from cases and controls were resistant to the commonly-used drugs. Thirteen of the 121 strains were phage-typeable and belonged to the phage type 193. However, no clinical or epidemiological significance could be established with these typeable strains. The findings of the study indicate that diarrhoeagenic *Salmonella* is one of the major pathogens causing diarrhoeal diseases in eastern India.

Key words: *Salmonella enterica*; Diarrhoea; Microbial sensitivity tests; Antibiotic resistance; Drug resistance; Microbial; Bacteriophage typing; Serotyping; India

INTRODUCTION

Salmonella enterica serotypes are an important enteric pathogen throughout the world, and the infection caused by this organism is endemic in India (1,2). Of 130 reported *S. enterica* serotypes, *Salmonella Typhimurium* is the most common one (3). This serotype not only causes sporadic diarrhoea in the community but is also responsible for outbreaks in many paediatric hospitals in India (4).

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Although *S. enterica* serotypes are an important aetiological agent of acute diarrhoea in developing countries, its isolation rate never exceeded 4-5% in the Calcutta region, and only one serotype—*S. Typhimurium*—could be recovered during those study periods (5,6). Therefore, this study was undertaken to investigate the prevalence of serotypes, antimicrobial susceptibility, and phage typing of *S. enterica* serotypes. Clinical presentations of the infected cases were also studied.

MATERIALS AND METHODS

Patients

The study was conducted during August 1993-September 1996. First two children of both the sexes, aged up to two years, with acute watery diarrhoea of less than three

days, admitted to the Diarrhoea Treatment and Training Unit of Dr. B.C. Roy Memorial Hospital for Children, between 8.00 a.m. and 1.00 p.m. during the first four days of the week, were initially enrolled in the study by an attending physician. Acute diarrhoea was defined as passage of three or more watery stools during the preceding 24 hours. The children who received antibiotics or antiparasitic drugs before hospitalization and also before collection of samples and who had other systemic illnesses were excluded from the study.

Controls

Children who were not suffering from diarrhoea for the preceding two months but attending the hospital for vaccination or nutritional rehabilitation were included in the study as controls. Children were matched for age, sex, nutritional status, and socioeconomic status. The control children who were receiving antibiotics or antiparasitic drugs were also excluded from the study. These children were not followed up to detect any signs and symptoms of infection due to *Salmonella* species.

Clinical evaluation of patients

On enrollment, a detailed clinical history was obtained from the parents, and thorough physical examinations were performed. Children were weighed in a balance of 20-g precision. The clinical manifestations regarding the nature and frequency of diarrhoea, frequency of vomiting, abdominal pain, pyrexia, cough and coryza, convulsion, and degree of dehydration were recorded in a standard proforma.

Stool collection

Unformed stool specimens, produced at the time of enrollment, were collected from children with diarrhoea in a sterile McCartney's bottle using sterile catheter and were transported immediately to the laboratory for evaluation. Sterile plastic containers were supplied to the mothers of control children with the advice to collect freshly-passed stools in the next morning and to deposit for evaluation.

Microbiologic procedure

The faecal samples were pre-enriched in selenite F broth at 37 °C for 18 hours. The enriched samples were plated in different selective media, namely bismuth sulphate agar (Difco), brilliant green agar (Difco), xylose lysin deoxycholate agar (Difco), *Salmonella*-*Shigella* agar (Difco), and Hekton enterica agar (Difco). A standard

method was used for characterizing suspected colonies (7). All the suspected strains were tested by slide agglutination, using *Salmonella* polyvalent O and H antisera, for confirming their identity as *Salmonella*. All the *Salmonella* strains were further serotyped by "factor 4,5 : i:1,2" antisera to confirm *S. Typhimurium* isolates. The remaining untypeable *Salmonella* strains were sent to the National *Salmonella* and *Escherichia coli* Reference Centre, Central Research Institute, Kasauli, Himachal Pradesh, for serotyping.

All the faecal samples were further cultured by conventional procedures for other bacterial pathogens, except *Yersinia enterocolitica*, *Campylobacter jejuni*, and *Helicobacter pylori* (7). Isolated *Escherichia coli* strains were further characterized to identify enteropathogenic (8) and enterotoxigenic *E. coli* (9) and were not further characterized to identify other types of *E. coli*, such as enteroaggregative, enterohaemorrhagic, or enteroadherent *E. coli*. *Entamoeba histolytica* and *Giardia lamblia* were identified by direct microscopic examination for presence of trophozoites and cysts by means of Lugol's iodine wet mount preparations. Oocytes of *Cryptosporidium* spp. were identified by modified acid fast-staining techniques of the Zeihl-Neelsen method. An enzyme-linked immunosorbent assay procedure was performed for the detection of rotavirus antigen in faeces (stored at -20 °C) as recommended by WHO (10).

Antimicrobial susceptibility and minimum inhibitory concentration testing

Antimicrobial susceptibility of all the isolated strains of *S. enterica* serotypes were done by the disc-diffusion technique using commercially-available antibiotic disc (Span Diagnostic Laboratory, India) as recommended by Bauer *et al.* (11). Reference strains of *E. coli* and *Pseudomonas*, susceptible to all the drugs, were used as controls. Strains, showing resistance to different antimicrobials, were tested for minimum inhibitory concentration (MIC) by the agar plate dilution method (12). MIC value more than 6.5 mg/L of resistant isolates was considered a break point.

Phage typing

Phage typing of all the isolated strains of *S. enterica* serogroups was done using 31 known phages at the Central Public Health Laboratory, Collindale, London and at the National *Salmonella* Phage Typing Centre, Lady Hardinge Medical College, New Delhi.

RESULTS

Faecal samples of the 1,025 children with acute watery diarrhoea were screened to detect enteropathogens. Specific aetiology could be detected in 653 (63.7%) cases as a single pathogen and in 106 (10.3%) cases as multiple pathogens. Rotavirus, enteropathogenic *E. coli*, and *S. enterica* serogroups were the most common enteropathogens isolated. *S. enterica* serogroups were detected as the sole pathogen in 157 (15.3%) samples (Table 1). Four different *S. enterica* serogroups were detected. *S. Typhimurium* was detected in 160 (70%) cases. *S. Senftenberg*, *S. Infantis*, and *S. Virchow* were detected in 28 (17.8%), 14 (8.9%), and 5 (3.2%) cases respectively.

Enteropathogen	Positive	
	No.	%
Rotavirus	160	15.6
Enteropathogenic <i>E. coli</i>	158	15.4
<i>S. enterica</i> serotypes	157	15.3
<i>V. cholerae</i> O1	60	5.8
<i>Shigella</i> spp.	51	5.0
<i>G. lamblia</i>	16	1.6
<i>Aeromonas</i> spp.	14	1.4
<i>E. histolytica</i>	12	1.2
<i>Cryptosporidium</i> spp.	12	1.2
Enterotoxigenic <i>E. coli</i>	8	0.8
<i>V. cholerae</i> non-O1	5	0.5
Multiple pathogens	106	10.3
Total	759	74.0

Eleven (3.2%) of the 338 faecal samples of the control children were positive for *S. Typhimurium*. The detection rate of *S. enterica* serotype as a single pathogen from diarrhoea cases was significantly ($p < 0.05$) higher than that of controls.

S. enterica serogroups were equally distributed in all the paediatric age groups. No specific age group was identified as a high-risk group. *S. enterica* was isolated throughout the year. However, like other enteric pathogens, a higher isolation rate was observed during the summer and the monsoon months (March to September). A similar trend in isolation rate was observed in the three-year study period.

Clinical features

Five percent of the children with salmonellosis had severe dehydration, 40% had some dehydration, and 55%

had no dehydration. Irrespective of the type of dehydration, vomiting and fever were the commonest presentations of children with salmonellosis. The majority (76.4%) of the children complained of diffused pain throughout the abdomen. All the children recovered within an average of four days having no sequelae. No deaths occurred in these children, none developed intussusception, and no surgical intervention was required. Clinical presentations of the children infected with *S. enterica* serogroups, rotavirus, and enteropathogenic *E. coli* were compared. A special feature of clinical presentation in salmonellosis cases was that the pain in abdomen was more frequently observed compared to rotavirus and EPEC infections. Fever in salmonellosis cases was as common as in rotavirus infection but much higher than in the enteropathogenic *E. coli*-infected cases. Status of dehydration in three groups was similar (Table 2).

Antimicrobial resistance patterns

Table 3 shows the antimicrobial resistance patterns of *S. enterica* serotypes. *S. Typhimurium* strains, isolated from cases and controls, were resistant to ampicillin, chloramphenicol, co-trimoxazole, furazolidone, gentamicin, nalidixic acid, and tetracycline. Other *Salmonella* serotypes, such as *S. Seftenberg*, *S. Infantis*, and *S. Virchow*, had a similar resistance pattern. However, all the *S. enterica* serotypes were uniformly (100%) susceptible to norfloxacin and ciprofloxacin.

MIC values of all the in-vitro resistant drugs to *S. enterica* serogroups ranged from 800 mg/L to 1,600 mg/L.

Phage typing

Thirteen of the 121 *S. Typhimurium* strains were phage-typeable. All belonged to the phage type 193 (PT 193) and were recovered from cases only. Rest of the strains isolated from cases and controls were untypeable.

DISCUSSION

The purpose of this study was to assess the importance of *S. enterica* serotypes in causation of childhood diarrhoea in and around Calcutta, India. In the present study, the isolation rate was considerably higher (15%) than before (4-5%) (5,6). Furthermore, the difference in isolation rates between the cases and the controls indicated the importance of this pathogen in causing the disease. Detection of enteropathogens throughout the

Table 2. Clinical presentation of diarrhoeal children infected with *S. enterica* serogroups, rotavirus, and enteropathogenic *E. coli*

Parameter	Children infected with		
	<i>Salmonella</i> (n=157)	Rotavirus (n=160)	EPEC* (n=158)
Pre-admission duration of diarrhoea, days (mean±SD)	2.0±0.5	2.1±0.4	1.8±0.4
Frequency of stool before hospitalization (mean±SD)	15±4.5	16±5	14±4.5
Vomiting; no. (%)	135 (86)	144 (90)	48 (30.4)
Number of vomiting before hospitalization/day (mean±SD)	15±5.0	18±6	6±3
Abdominal pain; no. (%)	120 (76.4)	48 (30)	31 (19.6)
Anorexia/refusal of food; no. (%)	70 (44.6)	68 (42.5)	29 (18.3)
Fever >37 °C; no. (%)	125 (79.6)	130 (81.3)	20 (12.6)
Dehydration status			
No; no. (%)	86 (54.8)	80 (50)	95 (60.1)
Some; no. (%)	63 (40.1)	67 (41.9)	59 (37.3)
Severe; no. (%)	8 (5.1)	13 (8.1)	4 (2.5)

Figures in parentheses indicate percentage
*Enteropathogenic *E. coli*

year indicates the continuous source of infection throughout the year.

Despite a large number of serotypes, only four serotypes, such as *S. Typhimurium*, *S. Seftenberg*, *S. Infantis*, and *S. Virchow*, were isolated from this part of the country which also corroborates the findings of other

since it is also reported from other countries (18). The clinical and epidemiological significance could not be drawn from detection of only 13 phage-typeable strains.

Although all the *S. enterica* serogroups were uniformly resistant to the commonly-used drugs, the strains were, however, susceptible to norfloxacin and

Table 3. Resistance patterns of *Salmonella* serotypes

Drug	No. (%) of drug-resistant strains of <i>Salmonella</i> serotypes				
	<i>S. Typhimurium</i>		<i>S. Seftenberg</i> (n=28)	<i>S. Infantis</i> (n=14)	<i>S. Virchow</i> (n=5)
	Cases (n=110)	Controls (n=11)			
Ampicillin	109 (99.1)	11 (100)	28 (100)	14 (100)	5 (100)
Chloramphenicol	67 (61.5)	10 (90.9)	27 (96.4)	13 (92.9)	5 (100)
Co-trimoxazole	85 (76.0)	11 (100)	28 (100)	11 (78.6)	4 (80)
Furazolidone	82 (75.2)	11 (100)	22 (81.5)	3 (21.4)	5 (100)
Gentamicin	76 (69.7)	9 (81.5)	27 (96.4)	11 (78.6)	5 (100)
Nalidixic acid	105 (96.3)	11 (100)	28 (100)	13 (92.9)	1 (20)
Tetracycline	106 (97.2)	11 (100)	28 (100)	14 (100)	5 (100)
Norfloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Figures in parentheses indicate percentage

studies in different geographical locations where a limited number of serotypes were also prevalent (13,14). The present study further confirmed that *S. Typhimurium* is the commonest serotype in most developing countries (15), including India (16). Phage type 193 (PT 193) of *S. Typhimurium* is the only phage type isolated in Calcutta, but it was also reported from other countries (17). Although the high percentage of *S. Typhimurium* strains is not typeable, it is not an unusual phenomenon

ciprofloxacin. By contrast, *S. Typhimurium*, isolated during the 1980s, was uniformly resistant, but other *S. enterica* serogroups were sensitive (19). The *Salmonella*-infected children with acute watery diarrhoea having no or some dehydration were treated with oral rehydration solution only and without any antibiotics as this infection is usually self-limiting, and treatment with antimicrobial is controversial because of prolonged carriage and development of resistance. However, the severely-

dehydrated children received intravenous infusion of Ringer's lactate solution and ciprofloxacin therapy orally assuming that they were suffering from cholera, as Calcutta is endemic for this disease.

The present study clearly demonstrated that *S. enterica* serotypes were the most common infective agent and ranked third in the list of sole pathogen for secretory diarrhoea in children in this geographical location.

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