

OCCURRENCE OF MULTI-RESISTANT *Salmonella typhimurium* INFECTION IN A PEDIATRIC HOSPITAL AT CALCUTTA

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ABSTRACT

Salmonella typhimurium was isolated from 55 (15.9%) of the 347 hospitalized diarrheal children and 14 (11.1%) of the 126 non-diarrheal controls. All the 98 asymptomatic children attending hospital outpatients were negative. Six (3.7%) of the 162 samples of different categories examined from the hospital were positive for *S. typhimurium*. Finger washing of one female food handler, feces of two cats of the wards, surface of wash basin, lavatory seat and shelf yielded positive isolations. In vitro antibiotic sensitivity testing showed majority of the strains were resistant against commonly used antimicrobial agents while they were uniformly sensitive to norfloxacin and ciprofloxacin. The present study points to cross-infection by multi-resistant *S. typhimurium* strains in the hospital wards.

Key words: *S. typhimurium*, Prevalence, Multi-drug resistance, Cross infection.

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Salmonellosis has been recognized as one of the major public health problems in many parts of the world and *S. typhimurium* is the most common serotype encountered in the most geographical situations(1-4). Outbreaks of gastroenteritis caused by *S. typhimurium* have also been reported in many pediatric wards in different parts of India(5-8). Earlier studies conducted in Calcutta showed a fairly low incidence of *Salmonellae* among the diarrhea causing organisms particularly in hospitalized infants and young children(9). However, recently there has been a sudden outbreak of *S. typhimurium* gastroenteritis in the nursery of a general pediatric hospital in Calcutta (unpublished observations).

The present study was undertaken to determine the prevalence of *S. typhimurium* infection in hospitalized diarrheic children and also to explore the possibility of nosocomial spread in the same pediatric hospital.

Material and Methods

Collection of Specimens

Stool specimens from 347 infants and children admitted to the Dr. B.C. Roy Memorial Hospital for Children, Calcutta from July, 1988 to March, 1990, with acute diarrhea of less than four days' duration were investigated. Patients in the age-group 1 day to 5 years attending the hospital between 8-12 a.m. and without any prior antibacterial treatment were included for the study. The patients were treated in different general medical wards of the hospital along with other patients.

Samples of stool were collected from age and sex matched control of 126 non-diarrheic children hospitalized for a wide variety of illness and housed in common

wards along with the diarrhea patients. Fecal samples were also collected from 98 healthy babies attending the Out-patients Department for immunization and Well Baby Clinics without history of any diarrhea at the time of sampling as a second control.

In order to find out the mode of spread of the pathogen, 162 samples were also collected from stool and finger washings of medical and paramedical staff, other hospital articles and from animals frequently seen in the hospital wards. All the samples were transported to the laboratory within one to two hours of collection.

Isolation and Identification

All the fecal samples were directly inoculated onto bismuth sulphite and brilliant green selective agar plates and into a tube of selenite enrichment broth (Difco Laboratories, USA) and incubated at 37°C for 18 to 24 hours. After enrichment, the selenite broth cultures were further plated on selective media and incubated at 37°C for 18 to 24 hours. The food and environmental samples were at first pre-enrichment in buffered peptone water at 37°C for 18-24 hours(10) and subsequently enriched in selenite broth at 42°C for 18 to 24 hours(11) followed by plating onto selective agars. Water samples were filtered through millipore filter membrane of pore size 0.22 μ (Millipore Corporation, USA). The filter membranes were inoculated in buffered peptone water and further processed in the same manner as described above. Typical *Salmonella* like colonies were picked from each plate, characterized biochemically and confirmed by serogrouping with slide agglutination using specific antisera (Wellcome Reagents Limited, England)(12).

Drug Sensitivity Testing

Drug sensitivity testing was performed on Mueller-Hinton agar plates following the standard disc diffusion technique(13). The following antibiotic impregnated discs were used: amikacin (Ak), 30 mcg; ampicillin (I), 10 mcg; chloramphenicol (C), 30 mcg; ciprofloxacin (Ci), 5 mcg; furazolidone (Fz), 100 mcg; gentamicin (J), 10 mcg; nalidixic acid (Na), 30 mcg; norfloxacin (Nf), 10 mcg; tetracycline (T), 30 mcg; trimethoprim-sulphamethoxazole (Q), 25 mcg (Span Diagnostics, India). The reference strain *Escherichia coli* (ATCC 25922) sensitive to all the drugs used in the study, was routinely included. Interpretation of readings as sensitive, partially sensitive or resistant was made according to the chart(13). In this study, partially sensitive readings were few and therefore, considered as sensitive for the purpose of analyzing the data.

Phage Typing

Several strains of *S. typhimurium* isolated in this study were sent to the National Salmonella Phage Typing Centre, Lady Hardinge Medical College, New Delhi for confirmation and phage typing.

Results

Detection of *S. typhimurium* in children of different age groups is presented in Table I. The organism was isolated from 55 (15.9%) of the 347 hospitalized diarrheal children and 14 (11.1%) of the 126 non-diarrheal controls from hospital in-patients. None of the 98 asymptomatic control children from hospital outpatients yielded a positive isolation.

Of the 162 different categories of

TABLE I—Detection of *Salmonella typhimurium* in Children of Different Age groups

Age groups (mo)	No. of diarrhea cases		No. of hospitalized non-diarrheal controls		No. of control children attending hospital out-patient department	
	Screened	Positive (%)	Screened	Positive (%)	Screened	Positive (%)
0-6	130	28 (21.5)	30	3 (10.0)	18	Nil
7-12	112	15 (13.4)	24	1 (4.2)	16	Nil
13-24	54	5 (9.3)	37	6 (16.2)	26	Nil
25-36	23	4 (17.4)	17	2 (11.8)	17	Nil
37-48	15	2 (13.3)	10	Nil	10	Nil
49-60	13	1 (7.7)	8	2 (25.0)	11	Nil
Total	347	55 (15.9)	126	14 (11.1)	98	Nil

samples examined from the hospital, 6 (3.7%) were positive for *S. typhimurium*. Interestingly, the organism could be isolated from one female food handler who was responsible for distribution of food to the patients. The organism could also be isolated from feces of two pet cats of the wards, surface of wash basin, lavatory seat and shelves (Table II).

Drug susceptibility testing of the 55 *S. typhimurium* strains isolated from diarrhea cases showed 17 resistance (R) patterns. The commonest was C, Fz, I, J, Na, Q, T (30.1%) followed by Ak, C, Fz, I, J, Na, Q, T (25.5%). Among the 14 *S. typhimurium* strains isolated from non-diarrheal controls, 8 R-patterns were noted. The commonest was Ak, C, Fz, I, J, Q, T (42.9%). Similarly, of the 6 isolates from hospital environment 4 R-types were discernible with the commonest R-types being C, Fz, I, J, Na, Q, T and Ak, Fz, I, J, Na, Q, T (33.3% each).

Of the total 75 isolates of *S. typhimurium* from different categories of samples, 31 strains were phage typed and all were found untypable.

Discussion

In this study a distinct high isolation of *S. typhimurium* causing diarrhea amongst hospitalized infants and children was observed. The earlier study during 1982-83 on etiological spectrum of acute diarrhea conducted in the same children's hospital revealed only 0.9% isolation of *S. typhimurium* while rotavirus (13.8%) and *Shigella* species (12.1%) were found as dominant etiologic agents(9). A similar increasing frequency of detection of *S. typhimurium* has also been observed in a hospital based study conducted at Vellore, South India, which showed 1 isolation in 1977, 20 in 1978, 66 in 1979 and 146 in 1980(7).

Overall isolation of this organism among hospitalized non-diarrheal controls (11.1%) was almost similar to that of diarrhea cases (15.9%) admitted to the pediatric wards from different parts of the community. Whereas, none of the control children attending hospital Outpatients Department were positive for this organism. Such contrasting isolation rates

TABLE II—Isolation of *S. typhimurium* Strains from Different Categories of Samples from B.C. Roy Memorial Hospital for Children, Calcutta

Source	Category	No. sampled	No. positive for <i>S. typhimurium</i>
Human	Stool samples from doctors & paramedics	17	Nil
	Finger washing from doctors & paramedics	35	1 (2.9)
Animal & insect	Rat stool	7	Nil
	Cat stool	8	2 (25.0%)
	Cockroaches	5	Nil
	Flies	6	Nil
Food	Cooked/raw including milk	11	Nil
Environmental	Tap/stored water	13	Nil
	Wash basin	8	1 (12.5%)
	Lavatory seats	6	1 (16.7%)
	Furniture	8	1 (12.5%)
	Swab from floor	14	Nil
	Bed clothes and mattresses	10	Nil
	Equipments	10	Nil
	Handle of doors/ refrigerator	4	Nil
Total		162	6 (3.7%)

clearly indicate a nosocomial spread of infection of *S. typhimurium* in the pediatric wards of the hospital. Hospital cross-infection was inevitable since diarrheic patients were treated in the same ward along with the other patients suffering from varied illnesses. It was further observed that the organism was persisting in the different medical wards which was substantiated by the isolation from a female food handler, pet cats in the ward, wash basin, lavatory seats and shelves of the hospital. Moreover, the excretion of *S. typhimurium* from in-patient controls indicate the high circulation of the organism in the wards. It was also noticed that more than a dozen cats were living in the different medical wards and sharing the left over foods of the patients. Results of

the present study confirmed that apart from diarrhea patients and finger washing positivity the other source of infection may be the infected cats. The presence of *S. typhimurium* in animals, water, sewage and environmental samples have also been reported by Saxena and co-workers(2).

An outstanding feature of the *S. typhimurium* strains from human sources were multiple drug resistant to commonly used antimicrobial agents which was in agreement with results documented by other workers(4,6,7). It was interesting to note that even the strains isolated from hospital environment were also resistant to a number of antimicrobial agents in the present study. In contrast, multiple drug resistant strains were not encountered in the *Salmonellae* isolated from hospital

food and vermin in a study conducted in North India(14). However, all the strains isolated from different sources were sensitive to norfloxacin and majority (96.0%) to ciprofloxacin. But these antibiotics are not suitable for treatment in infants due to toxic effect of the drugs. Thus, increased drug resistance to antibiotics poses a significant problem, for the treatment of diarrhea particularly in infants developing complications by this organism. Moreover, it was difficult to make any definite conclusion regarding the mode of transmission of this organism by phage typing as well as antibiotic resistance patterns of the strains isolated from diarrhea cases, controls and hospital environments. However, it may be postulated that the same types of multi-resistant *S. typhimurium* isolates are circulating in the hospital environment and repeating the cycle of cross-infection to the susceptible host.

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