

SALMONELLA CARRIAGE RATE AMONGST SCHOOL CHILDREN - A THREE YEAR STUDY

SJN Devi¹ and CJ Murray²

¹Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA.

²Salmonella Reference Laboratory, The Institute of Medical and Veterinary Science, Adelaide, South Australia 5000.

Abstract. As a part of three-years epidemiological survey of salmonellosis in South Kanara District, a southwest coastal region of India, an attempt was made to determine the human *Salmonella* carriage rate during the period between 1981 to 1983. One thousand and two asymptomatic school children 5 to 15 years of age, 42 restaurant employees and 17 dairy workers formed the subjects of this bacteriological study. The *Salmonella* carriage rate among healthy school children from a single fecal sampling was 1% and no *Salmonellae* were recovered from the feces of restaurant and dairy workers. Ten school children excreted *Salmonellae* which belonged to 6 different serotypes-*Salmonella oslo*, *S. ohio*, *S. typhimurium*, *S. urbana*, *S. cerro* and *S. derby*; *S. oslo* and *S. ohio* were the most frequent serotypes. No-*S. typhi*, *S. paratyphi A* or *S. paratyphi B* were recovered. All *Salmonella* strains recovered during this study were dual or multiple drug resistant, sulfadiazine and tetracycline resistance being commonly observed. The significance of excretion of potentially pathogenic, drug resistant *Salmonellae* by symptomless school children is discussed from public health point of view.

INTRODUCTION

Despite the improvements over the past years, salmonellosis continues to be a problem of increased concern all over the world, developing countries in particular. Both animals and humans form the source of infection and outbreaks. Symptomatic and asymptomatic animals and humans contribute equally to the problem of salmonellosis. Emergence of drug resistance among *Salmonellae* has further complicated the problem. Human carriers act as potential sources of outbreaks in food poisoning caused by *Salmonellae* (Cruickshank and Humphrey, 1987). Surprisingly, estimations of nontyphoid *Salmonella* carriers in the general population are quite few. Edwards and co-workers (1964) suggested that, in the US, 2-5 persons per 1000 of the population might be *Salmonella* carriers. A survey in the United Kingdom in 1965 revealed that 0.15% of 25249 children under the age of 5 years excreted

Salmonella (PHLS and SMOH Report, 1965). The largest study done in Tokyo involved screening of 4462287 stools, of which 0.15% were positive for *Salmonella* (Sakai, 1978). Buchwald and Blaser (1984) have estimated that 200000 *Salmonella* excretors exist in the US population at any one time.

The incidence of childhood gastroenteritis due to *Salmonellae* ranges in India from 2-18% (Pavri, 1952; Misra and Gupta, 1954; Joseph, 1980). South Kanara District is located in the state of Karnataka in the southwest coastal region of India and comprises people of mixed socio-economic status. Enteric fever and gastroenteritis of *Salmonella* etiology occur throughout the year in this geographic area (Devi, 1989). A wide variety of *Salmonella* serotypes have been isolated from human, animal and environmental sources in this region of the country, *S. typhimurium* and *S. typhi* being the most predominant ones (Devi, 1989). Although the role of natural animal reservoirs in the transmission of salmonellosis is well established (Devi and Shivananda, 1983 b), the contribution of asymptomatic carriers to the incidence of salmonellosis has not been studied. The purpose

Address for correspondence and reprint request: Dr JN Sarvamangala Devi, LDMI, NICHD, National Institutes of Health, Bldg 6; Rm. 1A05, Bethesda, Maryland 20892, USA.

of the present study was to determine the *Salmonella* carriage rate in healthy school children, restaurant and dairy workers and to study the serotype distribution and the antibacterial drug resistance pattern of isolated *Salmonellae*.

MATERIALS AND METHODS

Subjects

A single fecal sample was collected from a total of 1002 school children from 7 different schools distributed in the urban, suburban and rural areas of South Kanara District during January, 1981 through December, 1983. These school children were in the age range of 5-15 years; there were 480 boys and 522 girls. The children were asked about having present or recent diarrheal symptoms (in the past 4 months). During this 3 years period, a single fecal sample also from 42 restaurant employees and 17 dairy workers was analysed bacteriologically for the presence of *Salmonellae*. The restaurant and dairy employees were all males, between 18 to 36 years of age.

Collection and processing of fecal specimens

The stool samples collected in sterile vials were transported to the laboratory within 2-4 hours after collection. The samples were plated out directly and after enrichment, on MacConkey's agar, eosin-methylene blue agar and brilliant green agar; selenite F broth and tetrathionate broth (Cruickshank *et al*, 1975) were used as enrichment media and were incubated at 37°C for 24-30 hours. As enteric fever continues to be endemic in the area that was encompassed in this study, an attempt was made to screen the specimens also for enteric *Salmonellae* and therefore, Wilson and Blair's media were included as well. The culture plates were incubated at 37°C for 24-72 hours.

Identification of *Salmonella*

Salmonellae were identified by subjecting the strains to a battery of biochemical tests (Edwards and Ewing, 1972). The biochemical identification was confirmed by slide agglutination, tube agglutination and coagglutination tests (Devi and Shivananda, 1983a) with *Salmonella* poly 'O' and 'H' antisera, A to E. The final reconfirmation of identification and phage typing (Callow, 1959)

was done at the National Salmonella Center, Adelaide, Australia.

Estimation of number of *Salmonella* in feces

The procedure of McCall and co-workers (1966) was followed to determine the most probable number of *Salmonella* per gram of feces. Briefly, 10% fecal suspensions were prepared in tetrathionate broth, from which ten-fold dilutions were made. The broth dilutions were plated out on brilliant green agar plates, incubated for 24-48 hours and *Salmonellae* were identified as described above.

Antibiotic sensitivity test

Kirby-Bauer's high content single disc diffusion technique (Bauer *et al*, 1966) was used to examine the resistance (R) pattern of isolated *Salmonellae* to 11 antibacterial agents - ampicillin (A, 10 µg/ml), chloramphenicol (C, 30 µg/ml), furadantin (Fu, 300 µg/ml), gentamicin (G, 10 µg/ml), kanamycin (K, 30 µg/ml), streptomycin (S, 10 µg/ml), sulphadiazine (Su, 300 µg/ml), tetracycline (T, 30 µg/ml), co-trimoxazole (Tm, 25 µg/ml), nalidixic acid (Nx, 30 µg/ml), and polymixin B (Pb, 300 units/ml).

RESULTS

The bacteriological survey among 1002 school children of age range 5-15 years revealed that 1 out of every 100 school children excreted *Salmonella* and none of them gave a history of present or recent diarrheal symptoms (in the past 4 months). The children who carried *Salmonella* were between 6 to 10 years of age; no sex predominance in *Salmonella* carriage was observed. The strains belonged to 6 *Salmonella* serotypes - *S. oslo*, *S. ohio*, *S. typhimurium*, *S. derby*, *S. cerro* and *S. urbana*; *S. oslo* and *S. ohio* were the commonest serotypes. The single isolate of *S. typhimurium* was untypable. All *Salmonella* isolates were resistant to one or other antimicrobial drug used in this study; SuT was the commonest R-pattern observed. The number of *Salmonella* excreted per gram of feces varied from 11 to 1100 in all excretors, except the one who harbored *S. typhimurium*; the latter excreted ~ 10⁵ *Salmonellae* per gram of feces (Table 1).

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Table 1

Salmonella serotypes recovered from healthy school children, estimated fecal content and R-patterns. R-patterns.

Number studied [#]	No. culture positive (Sex-Age [years])	Serotype	R-pattern	Colonies/g of feces
92	1 (Male-8)	<i>S. derby</i>	SuNxPb	160
177	1 (Male-6)	<i>S. typhimurium</i>	TNx	110,000
179	1 (Male-10)	<i>S. cerro</i>	TNx	17
204	1 (Female-7)	<i>S. oslo</i>	SuNx	460
	1 (Female-7)	<i>S. urbana</i>	FuSuT	15
193	1 (Male-7)	<i>S. oslo</i>	SuT	150
	1 (Male-8)	<i>S. oslo</i>	SuTTm	240
69	1 (Female-8)	<i>S. ohio</i>	SuT	39
	1 (Female-10)	<i>S. ohio</i>	SuT	48
88	1 (Female-7)	<i>S. oslo</i>	SuT	1,100

[#] Children from 7 different schools.

No *Salmonellae* were recovered from the feces of 42 restaurant and 17 dairy workers. *S. typhi*, *S. paratyphi* A or *S. paratyphi* B were not isolated from any group of subjects included in this survey.

DISCUSSION

The prolonged fecal excretion of *Salmonella* is a well established sequela of intestinal salmonellosis. In the present study, 1% of 1002 school children screened were symptom-free excretors of *Salmonella*, as revealed by the culture of a single fecal sample. This figure is comparable to (Sen, 1968) or lower than those reported by others in the general population from different parts of India (Makholia and Singh, 1964; Datta and Singh, 1964; Singh and Kaura, 1976;). The children included in this study came from mixed socio-economic community. The *Salmonella* isolates were represented by 6 different serotypes with differing patterns of antibiotic resistance. From the isolation frequency of certain serotypes, such as *S. oslo* and *S. ohio* and the occurrence of similar R-patterns ie SuT, it is tempting to speculate about the possible occurrence of small, regional epidemics of nontyphoid *Salmonella* infections. The chain of transmission of *Salmonellae* in a community is often complex. Many outbreaks of salmonellosis go unnoticed or unreported in

endemic areas because of less severity of the symptoms. It is more likely that school children acquire *Salmonella* from the contaminated environment, suffer from self-limited gastroenteritis and continue to shed the bacteria for varying periods of time. Recovery of drug resistant *Salmonellae* from school children of this endemic area was not surprising. As revealed by a three-year epidemiological study, there was a constant circulation of drug resistant *Salmonellae* in the community, both among humans and animals, and in the environment (Devi, 1989; Devi and Shivananda, 1983). This is most likely due to the uncontrolled availability of antibiotics over the pharmacy counters and the indiscriminate use of antibiotics in human and animal population.

The duration of *Salmonella* excretion is influenced by age. Several workers have screened the victims of major nontyphi *Salmonella* outbreaks to determine the duration of fecal excretion (Buchwald and Blaser, 1984; Rubenstein *et al*, 1944; Szanton, 1957). While 2.6% of children less than 5 years of age excreted nontyphoid *Salmonella* (Buchwald and Blaser, 1984), the incidence of excretion was only 0.3% among 5 to 14 year-old children (Ames and Robins, 1943). A study of a *S. montevideo* outbreak showed that children of age 2 years and above, more often remained asymptomatic compared to younger children (Silverstolpe *et al*, 1961). The age of culture-positive school

children in this study ranged between 6-10 years; both male and female school children equally carried *Salmonella* in their feces.

The prolonged excretion of *Salmonella* is more common in patients with symptomatic salmonellosis than in those that are asymptomatic (Buchwald and Blaser, 1984). Children are known to excrete *Salmonella* in larger numbers (McCall *et al*, 1966) and for longer periods than adults (Cruickshank and Humphrey, 1987; Schothorst and Beckers, 1978). The median duration of *Salmonella* excretion after initial infection has been estimated to be 5 weeks (Buchwald and Blaser, 1984). The school children included in this survey, by themselves or through the assistance of their parents, failed to recall any history of diarrheal symptoms, 4 months prior to the collection of the fecal samples.

The carriers of *Salmonella* are known to excrete the pathogen intermittently. The rate of detection of *Salmonella* depends on the number of fecal samples obtained and the culture technique used. Cultures of 2 consecutive fecal specimens have detected 100% of short-term carriers and 87% of long-term carriers (McCall *et al*, 1966). During this survey, requests for collection of consecutive fecal samples were turned down by either parents or school administration. Although this refusal limited our study to a certain extent, excretion of drug resistant *Salmonella* by asymptomatic school children reflects the contribution of these subjects to the occurrence and perhaps persistence of *Salmonellae* in and around South Kanara District.

Food handlers and dairy workers are categorized as 'high-risk groups' of *Salmonella* carriers (Walker and Jones, 1987). The screening of a single fecal sample from a limited number of subjects of these 2 occupations did not reveal any *Salmonella* carriage. Chronic carriage of *S. typhi* for a duration of 12 months or more has been noted in 2-4% of typhoid patients (Buchwald and Blaser, 1984). During the three years of this investigation, no *S. typhi* carriers were detected. This was an unusual observation for this area, where enteric fever due to *S. typhi* and *S. paratyphi A* occurs throughout the year (Devi, 1989). However, this may not represent the real situation, because our attempts to screen several consecutive fecal samples were unsuccessful.

Morbidity and mortality due to septicemia and

gastroenteritis caused by nontyphi *Salmonella* is enormous, particularly in regions where the disease is endemic. The contribution of asymptomatic carriers to the occurrence of outbreaks of nontyphoid salmonellosis is described to be insignificant (Cruickshank and Humphrey, 1987). In a 10-year study of 396 outbreaks of *Salmonella* food poisoning in England and Wales, asymptomatic *Salmonella* excretors were implicated only in 0.5% of the outbreaks (Roberts, 1979). Considering the fact that initiation of infection requires only a low dose of *Salmonella* (Blaser and Newman, 1982; D'Aoust and Pivnick, 1976), excretion of even $\sim 10^1$ to 10^3 *Salmonella* by 90% of culture-positive healthy school children becomes significant, especially in an endemic locality. Different serotypes recovered in this study acquire importance because of their drug resistance, invasiveness and frequent isolation from human, animal and environmental sources in and around South Kanara District and other parts of the country (Devi, 1989; Devi and Shivananda, 1983b). The risk of transmission to other school children, younger and older family members is often great in settings where there is poor hygiene. The knowledge of the *Salmonella* carrier rate in a community helps in implementation of optimal hygienic and preventive regimens. Our study points to the practical significance of the fecal carriage of drug resistant *Salmonella* by asymptomatic school children and to the degree of risk that such subjects may pose to the community.

ACKNOWLEDGEMENTS

The first author acknowledges the laboratory facilities provided by Dr PG Shivananda, Department of Microbiology, Kasturba Medical College, Manipal, India. The co-operation of the parents and the principals of all schools who permitted to include their school children in this survey is gratefully acknowledged.

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