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Shigella infections among children in Andaman – an archipelago of tropical islands in Bay of Bengal

A. R. GHOSH AND S. C. SEHGAL*

Regional Medical Research Centre (*Indian Council of Medical Research*), *Port Blair-744 101, Andaman & Nicober Islands, India*

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SUMMARY

Shigellosis is common among children in the Andaman and Nicobar islands. Our experience showed two distinct features of shigellosis within a span of 3 years in 1994–6: (i) changing patterns of serotype or subtype specific shigellosis and (ii) emergence of multidrug resistant isolates with changing R-patterns. The rate of isolation was 10·4–27·9% with the rate of isolation of *Shigella flexneri* interchanging with *S. dysenteriae* alternately. In 1994, *S. flexneri* superseded *S. dysenteriae* (48·6% vs. 33·3%; $P < 0{\cdot}05$) while *S. dysenteriae* dominated over *S. flexneri* in 1995 (54·7% vs. 34·0%; $P < 0{\cdot}05$). The picture reversed again in 1996 (63·0% vs. 22·2%; $P < 0{\cdot}05$). Among shigellae isolates, the commonest serotypes were *S. dysenteriae* type 1 and *S. flexneri* type 2a. Isolated shigellae were of multidrug resistant type. Seven R-patterns were observed in 1994, while 8R-patterns were observed during the next year with the emergence of nalidixic acid resistance. In 1996, emergence of gentamicin resistance was also observed. All isolates were resistant to ampicillin and sensitive to quinolones. The MIC of nalidixic acid and gentamicin are $\geqslant 128\ \mu$g/ml and $\geqslant 64\ \mu$g/ml respectively. These changing trends in shigellosis has important public health significance.

INTRODUCTION

Diarrhoeal diseases claim the lives of at least five million children per year in the developing countries including India [1] and shigellosis or bacillary dysentery is responsible for approximately 10% of these deaths [2]. The attack rate of shigellosis in India is 1–15% [3]. Acute diarrhoea among children is one of the most commonly observed clinical entities in the Andaman and Nicober Islands but information about the etiology and drug sensitivity pattern of isolates is lacking due to the lack of diagnostic facilities and their remote location. The islands attract a number of visitors from the mainland and from abroad every year. Hence, there remains always a possibility of introduction of new pathogens or unusual clones of the enteric pathogens into the existing flora in these tropical islands. Recent investigation on acute childhood diarrhoea and even outbreaks revealed the fact that shigellosis is the most common diarrhoeal disease in the islands [4, 5].

Besides the promotion of improved personal hygiene, a measure designed to decrease the prevalence of all enteropathogens, the current control measures for shigellosis consists of antibiotic therapy alone [6]. Unfortunately, the options for antimicrobial therapy of shigellosis have narrowed considerably in recent years as bacterial resistance has increased. Nalidixic acid, to which most of the strains were sensitive, was considered to be the drug of choice [7]. However, the resistance of shigella strains to nalidixic acid has

* Author for correspondence: Prof. S. C. Sehgal, Director, Regional Medical Research Centre (I.C.M.R.), Post Bag No. 13, Port Blair – 744 101, Andaman & Nicobar Islands, India.

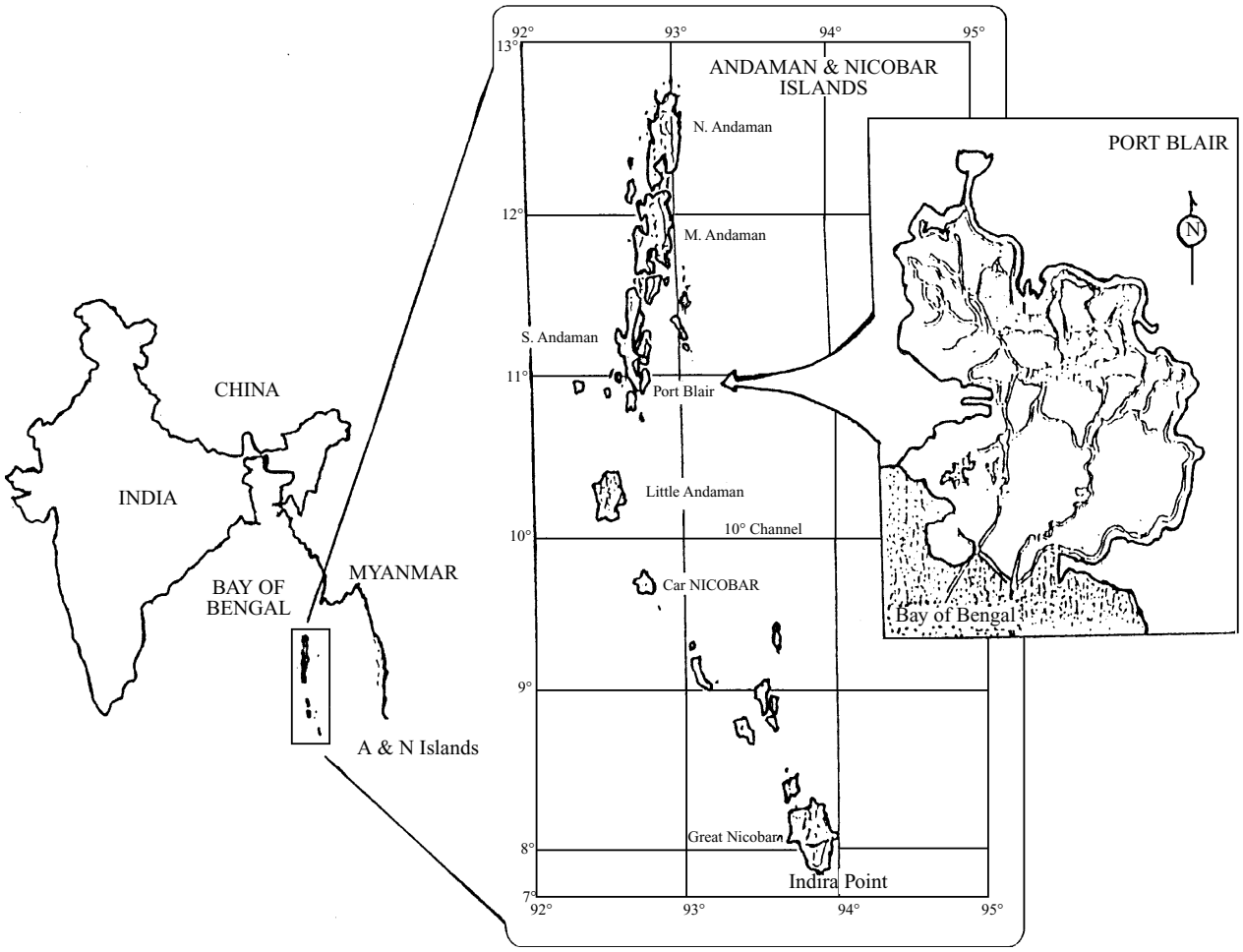

Fig. 1. Map of Andaman and Nicobar Islands and insert picture showing Port Blair, the study area.

already been documented from several parts of the world including India [8–10].

We had been observing a very high resistance to ampicillin and co-trimoxazole in the past but 100% sensitivity to nalidixic acid was noticed till recently. In the present study, we report the results of 3 years surveillance on shigellosis and compare the situation in these islands with that of other parts of south east Asia.

MATERIALS AND METHODS

Study area

Andaman and Nicober islands, a Union Territory of India, comprise an archipelago of 321 islands in the Bay of Bengal, lying in a vertical line within 92° to 94° East longitude and between 6° to 14° North latitude. The mountain ranges which form the Himalayan chain north of India, on the eastern side seem to curve upwards into China and downwards forming the mountain barriers between India and Myanmar. The extension of these ranges continues through the Andaman and Nicobar islands and thereafter further east into Indonesia (Fig. 1). Port Blair, the capital town in the Andaman district, can be reached from the mainland (Calcutta and Chennai; approximately 1200 km away by air and sea) and other islands by regular ferry services. G. B. Pant Hospital, Port Blair is the only referral hospital in the islands and has 408 beds. Approximately 750 diarrhoeal cases report to paediatric out-patient department annually.

Specimens and methods

A total of 1079 faecal specimens were studied during the period January 1994 to December 1996, from diarrhoeic children aged below 5 years admitted to the G. B. Pant Hospital, Port Blair. During 1994, diarrhoeic patients who attended the out-patient department (OPD) were also included in this study. We observed in 1994 that the majority of patients had

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taken one or more antibiotics before coming to the OPD and the rate of isolation of shigellae was relatively low among those cases. Since shigellosis is a relatively serious disease as compared to other diarrhoeal diseases and often requires hospitalization, we confined our study to the cases who had not taken antibiotics at least a week prior to the admission during 1995 and 1996.

Bacteriological procedures

Faecal samples were collected in Cary-Blair transport medium and were transported to the laboratory. Specimens were inoculated onto MacConkey agar (Oxoid), Hektoen Enteric agar (Oxoid), and Desoxycholate Citrate agar (Oxoid) and were incubated at 37 °C for selective isolation of shigellae. The plates were examined after 18–24 h of incubation. Suspected colonies were inoculated into Triple sugar iron agar (Oxoid), Mannitol motility medium (Hi-media, India), Urea medium (Hi-media), peptone water (1% Bactopeptone, Difco; pH 7·2) for biochemical identification of shigellae. Biochemically identified shigellae were confirmed by serotyping.

Serotyping and antibiotic sensitivity

Shigella serotypes were identified using standard commercially available antisera (Denka Seiken Co. Ltd, Tokyo, Japan). Antibiotic sensitivity was performed by disk diffusion technique [11]. The minimum inhibitory concentration (MIC) of shigellae against different antibiotics (Sigma Chemical Co., USA) like nalidixic acid, gentamicin, streptomycin, tetracycline and ampicillin was determined by agar dilution method [12].

Statistical analysis

Data were analysed using Epi-Info [13] and discrete variables were compared by the χ^2 test.

RESULTS

Between January 1994 and December 1996, a total of 1079 diarrhoeic children were bacteriologically analysed for shigellosis; 152 (14·1%) had shigella infection. Yearwise, *Shigella* spp. was isolated from 72 of 691 diarrhoeic children (10·4%), 53 of 190 patients (27·9%) and 27 of 198 patients (13·6%) during 1994, 1995 and 1996, respectively. The serotype distribution of shigellae (each isolate representing a case) is shown in Table 1.

Table 1. *Distribution of* Shigella *species and serotypes during 1994–6 in Port Blair*

	Years		
Isolates	1994	1995	1996
Sample examined	691	190	198
Shigella isolates	72 (10·4)*	53 (27·9)	27 (13·6)
S. dysenteriae	24 (33·3)	29 (54·7)	6 (22·2)
Type 1	18	24	4
Type 2	2	2	2
Type 7	4	3	0
S. flexneri	35 (48·6)	18 (34·0)	17 (63·0)
Type 1a	3	4	0
Type 1b	4	1	2
Type 2a	23	9	10
Type 2b	1	1	2
Type 3a	0	0	1
Type 6	2	2	2
Variant X	1	0	0
Variant Y	1	1	0
S. boydii	7 (9·7)	2 (3·8)	2 (7·4)
Type 7	3	0	1
Type 14	4	2	1
S. sonnei	6 (8·3)	4 (7·5)	2 (7·4)

* Figures in parentheses indicate percentages.

Among shigellae, *S. flexneri* was found to predominate during 1994 and 1996 while *S. dysenteriae* was the commonest *Shigella* spp. during 1995. Again type 2a of *S. flexneri* and type 1 of *S. dysententeriae* were isolated predominantly during the study period. Variation in serospecific infection was observed. Infection due to *S. dysenteriae* type 1 and *S. flexneri* type 2a shifted from year to year. During 1994 *S. flexneri* dominated over *S. dysenteriae* (48·6% vs. 33·3%; χ^2 at 2 D.F. is 8·93; $P < 0·05$) during 1995 *S. dysenteriae* superseded *S. flexneri* (54·7% vs. 34·0%; χ^2 at 1 D.F. is 4·64; $P < 0·05$)), and in 1996 *S. flexneri* again superseded *S. dysenteriae* (63·0% vs. 22·2%; χ^2 at 1 D.F. is 7·84; $P < 0·05$). Other serotypes involved in causing shigellosis were *S. dysenteriae* 2, 7; *S. flexneri* 1a, 1b, 2b, 3a, 6 and variant x and y; *S. boydii* types 7, 14 and *S. sonnei*.

Multiple antibiotic resistance was observed among the strains of shigella isolated. More than four commonly used antibiotics were ineffective against shigellae. The rate of susceptibility against drugs like co-trimoxazole and nalidixic acid decreased from 20·7 to 0·0% and 100·0 to 85·2% respectively from 1994

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Table 2. *Antimicrobial drug sensitivity of isolated shigella species*

Antibiotics	μg/disc	1994 ($n = 72$)	1995 ($n = 53$)	1996 ($n = 27$)	Total ($n = 152$)
Ampicillin (AM)	10	0 (0·0)*	0 (0·0)	0 (0·0)	0 (0·0)
Ciprofloxacin (CF)	5	72 (100·0)	53 (100·0)	27 (100·0)	152 (100·0)
Chloramphenicol (CH)	30	63 (87·5)	32 (60·4)	6 (22·2)	101 (66·40)
Co-trimoxazole (CO)	25	14 (19·4)	7 (13·2)	0 (0·0)	21 (13·8)
Gentamicin (GM)	10	72 (100·0)	53 (100·0)	17 (63·0)	142 (93·4)
Nalidixic acid (NA)	30	72 (100·0)	45 (84·9)	23 (85·2)	140 (92·1)
Norfloxacin (NF)	10	72 (100·0)	53 (100·0)	27 (100·0)	152 (100·0)
Nitrofurantoin (NT)	300	68 (94·5)	45 (84·9)	23 (85·2)	136 (89·5)
Streptomycin (ST)	10	6 (8·3)	4 (7·5)	0 (0·0)	10 (6·6)
Tetracycline (TE)	30	6 (8·3)	4 (7·5)	0 (0·0)	10 (6·6)

* Figures in parentheses indicate percentages.

Table 3. *Antimicrobial susceptibility of* Shigella dysenteriae *type 1 isolated during 1994–6 in Port Blair*

Drug used	1994 ($n = 18$)	1995 ($n = 24$)	1996 ($n = 4$)
Norfloxacin	18 (100·0)*	24 (100·0)	4 (100·0)
Ciprofloxacin	18 (100·0)	24 (100·0)	4 (100·0)
Gentamicin	18 (100·0)	24 (100·0)	3 (75·0)
Nalidixic acid	18 (100·0)	16 (66·7)	0 (0·0)
Nitrofurantoin	16 (88·9)	20 (83·3)	2 (50·0)
Chloramphenicol	14 (77·8)	15 (62·5)	0 (0·0)
Co-trimoxazole	4 (22·2)	2 (8·3)	0 (0·0)
Streptomycin	3 (33·3)	1 (4·1)	0 (0·0)
Tetracycline	2 (11·1)	1 (4·1)	0 (0·0)
Ampicillin	0 (0·0)	0 (0·0)	0 (0·0)

* Figures in parentheses indicate percentages.

through 1996 (Table 2). The antimicrobial susceptibility of *S. dysenteriae* type 1 during the study period is shown in Table 3. Interestingly, the nalidixic acid resistance property was restricted to *S. dysenteriae* type 1 only, while resistance to gentamicin was found evenly distributed among different shigellae isolated in 1996. All isolates were sensitive to nalidixic acid during 1994. In 1996, appearance of gentamicin resistance was observed among 37·0% (10 of 27) *Shigella* spp. As resistance to nalidixic acid and gentamicin emerged, a change in resistance (R) patterns was observed and this included 7 R-types during 1994, 8R-patterns during 1995 and 5 R-patterns during 1996 (Table 4).

The MIC value revealed that more than 90% of *Shigella* spp. isolated were resistant to ampicillin, tetracyline and streptomycin (Table 5). Isolates were moderately resistant to gentamicin and nalidixic acid. Eight isolates of *S. dysenteriae* type 1 emerged as resistant to nalidixic acid during 1995 and four isolates in 1996; MIC for those isolates were $\geqslant 128$ μg/ml. Along with nine isolates of different serotypes, one isolate of *S. dysenteriae* type 1 recovered was found resistant to gentamicin. The MIC against gentamicin was $\geqslant 64$ μg/ml.

DISCUSSION

Shigellosis accounts for majority of cases of bacterial diarrhoeas in infants and young children in developing countries. However, authentic information from these islands is scanty except for an outbreak in 1986 when *S. dysenteriae* type 1 was found as the most common causative organism being positive in 43 of the total 47 cases of shigellosis. The remaining four (8·5%) isolates belonged to *S. flexneri* [4]. In the present study, incidence of shigellosis was 10·4–27·9% during the year 1994–6. Overall percentage of *S. flexneri* (46·0%) was higher than *S. dysenteriae* (38·8%). *S. flexneri* was the predominant isolate during 1994 (48·6%) and 1996 (63·0%) respectively whereas *S. dysenteriae* was most frequently isolated in 1995 (54·7%). It may be due to the fact that there was an outbreak of dysentery in 1995 in Great Andamanese, a primitive tribe of these islands, where *S. dysenteriae* type 1 was isolated from 60·0% of the patients (unpublished). It has also been reported that *S. flexneri* is the predominant endemic species and may be responsible in approximately 50% of culture-positive cases [2, 14]. Epidemic potential of *S. dysenteriae* type 1 and endemic nature of *S. flexneri* have been known to occur in different parts of India including Karnataka, Kerala, Tamilnadu and West Bengal [15, 16]. Rahman and colleagues had similar observations in Dacca, Bangladesh [17]. *S. dysenteriae* type 1 has epidemic potential, causes

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Table 4. *Drug resistance patterns of shigella isolates during 1994–6*

Resistance pattern	1994 ($n = 72$)	1995 ($n = 53$)	1996 ($n = 27$)
AM	2 (2·8)*	1 (1·9)	—
AM, TE	4 (5·5)	3 (5·6)	—
AM, ST, TE	8 (11·1)	3 (5·6)	—
AM, ST, CO	4 (5·5)	3 (5·6)	—
AM, ST, TE, CO	41 (56·9)	14 (26·4)	5 (18·5)
AM, ST, TE, CO, NT	4 (5·5)	8 (15·1)	1 (3·7)
AM, ST, TE, CO, CH	9 (12·4)	13 (24·5)	4 (14·8)
AM, ST, TE, CO, CH, NA	—	8 (15·1)	4 (14·8)
AM, ST, TE, CO, CH, NT	—	—	3 (11·1)
AM, ST, TE, CO, CH, GM	—	—	10 (37·0)

* Figures in parentheses indicate percentages.

Table 5. *Minimum inhibitory concentrations (MIC) of different antimicrobials to shigella isolates*

Drugs	μg/ml		
	MIC_{50}	MIC_{90}	MIC range
Nalidixic acid	2·0	> 4·0	1·0–128·0
Gentamicin	4·0	> 8·0	1·0–64·0
Ampicillin	> 128·0	256·0	32·0–256·0
Tetracycline	128·0	> 128·0	4·0–> 128·0
Streptomycin	128·0	> 128·0	4·0–> 128·0

severe disease and has high case fatality rate [18]. However, *S. flexneri* 2a has been found to be the most common serotype worldwide and is responsible for majority of deaths due to shigellosis [19]. Though more than 45 serotypes of shigellae have been established, of them two stand out because of their special epidemiological behaviour in developing countries [18, 19]. It is pertinent to state that infection due to *S. flexneri* 2a has not been specifically reported so far from India except the present study. Reports on epidemic outbreaks of shigellosis in India are frequent but bacteriological investigations of even sporadic cases are often not being undertaken.

There has been an increase in the reported incidence of multidrug resistance *Shigella* spp., specially *S. dysenteriae* type 1 during the last decade or so [6]. Shigella isolates of 1986 outbreak in Port Blair was sensitive to gentamicin, nitrofurantoin, nalidixic acid and resistant to chloramphenicol, ampicillin, tetracycline and co-trimoxazole. The isolated shigellae in the present study are multiply resistant. In 1994, all shigella isolates were sensitive to nalidixic acid while resistance against this drug emerged in 1995 onwards. Gentamicin resistant isolates were also recovered during 1996. Emergence of nalidixic acid resistant shigella isolates has been well documented from different parts of India and elsewhere [3, 8, 9, 20]. Antibiotic resistance in shigellae has been a serious concern for clinicians and public health authorities. As Port Blair is connected with mainland India by regular ship and air services, a possibility of introduction of multiply resistant clones of *Shigella* spp. into these islands always remains. In absence of strict antibiotic policy in most of the developing countries and availability of antibiotics over the counter for self medication, there is always a danger of emergence of antibiotic resistance in otherwise sensitive bacteria in the community. The continuing emergence of drug resistant shigellae, particularly *S. dysenteriae* type 1 is narrowing considerably the efficacy of commonly used antibiotics in the treatment of shigellosis. Therefore, regular surveillance and monitoring of use of drugs may help to reduce this alarming increase in multiply resistance.

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