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EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF SHIGELLOSIS IN RURAL BANGLADESH

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Abstract

Some epidemiological and clinical characteristics of 292 patients infected with *Shigella* spp in a sample of 2,635 diarrhoea patients are described. The sample consisted of every fifth of all patients who came for free treatment between May 1983 and April 1984 at the Matlab Field Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh. A little more than half of them belonged to the age groups of 1-4 years (31.3%) and 5-14 years (20.4%), and more than half (56.4%) were males. Of the 292 patients yielding *Shigella* spp in their stools, the predominating isolates were *Shigella dysenteriae* type 1 (181; 62%) and *S. flexneri* (79; 27.1%) respectively. Of the stool samples from 1,318 patients examined for *Campylobacter*, 148 (11.2%) were culture-positive for only *Campylobacter jujuni*. *Shigella* spp were isolated most frequently from patients aged 1-2 years (154/1000), and in patients aged 60 years or more (147/1000), and least frequently in children under one year of age (58/1000). Compared with the genus as a whole, *S. dysenteriae* type 1 was isolated most frequently in patients aged 5-9 years (94/1000) and 10-14 years (116/1000) and more frequently from males (8.4%) than from females (4.9%; X^2=13.0; p<.001). Two peaks of shigellosis were observed, one during June-September caused by *S. dysenteriae* type 1, and the other in December caused by *S. flexneri*. Blood was visible to the naked eye in 68% of the faeces positive for *Shigella* spp (in 87% of faeces positive for *S. dysenteriae* type 1) on culture, but in only 18% of samples showing *Entamoeba histolytica*, in 18% of samples positive for *C. jejuni* on culture and 12% of samples from the remaining patients. Stool samples positive for *Shigella* spp on culture were more often alkaline than those from patients yielding other pathogens (251/276 vs 1580/2052; X^2=28.3; p<0.001). Patients infected with *S. dysenteriae* type 1 were more likely to have bloody stools than patients infected with other species of *Shigella* (150/181 vs 40/111; X^2=77.8; p<0.0001). Patients with shigellosis less frequently presented with moderate-to-severe dehydration (53/292 vs 1182/2343; X^2=108.9; p<0.0001), but required antibiotics more often (245/292 vs 1429/2343; X^2=58.8; p<0.0001). Patients infected with *S. dysenteriae* type 1 required antibiotics more frequently than those infected with other species of *Shigella* (166/181 vs 79/111; X^2=21.5; p<0.001). Salient findings, like the *S. dysenteriae* type 1 as the dominating isolate in place of *S. flexneri* observed earlier; two peaks of shigellosis, instead of one, known previously; similar value of the naked eye as well as the microscopic presence of blood in faeces as a diagnostic aid in shigellosis; antibiogram pattern of shigella isolates, etc., including their implications, are discussed.

Key words: Shigella; Dysentery, Bacillary; Epidemiology; Pathology; Diagnosis, Laboratory.

Introduction

As in most other developing countries, diarrhoea is a major cause of morbidity and mortality in Bangladesh, and shigellosis is of particular importance (1,2). To study some epidemiological and clinical characteristics of shigellosis we report here 292 patients infected with *Shigella* spp in a sample of 20% of patients seen during a period of

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one year at a rural hospital in Bangladesh. Some comparisons are made with the remainder of the sample and with patients infected with *Entamoeba histolytica* and *Campylobacter* spp, two other potential causes of dysentery.

Materials and methods

Background and *Surveillance Methods:* Since 1963, the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) has been providing free treatment for diarrhoea at a hospital at Matlab Bazar, a town in a rural riverine area of Bangladesh. At the time the study reported here was conducted, about 12,000 patients sought treatment every year. To study the causes and clinical characteristic of diarrhoea in the population served by the hospital, a surveillance system was instituted in 1983 to study in some detail every fifth patient. Between May 1st, 1983 and April 30 1984, 2,635 patients were studied, of whom 292 were found to be infected with *Shigella* spp.

For each subject, information on age and gender was recorded and a physician conducted an examination to record the signs and symptoms of disease and the state of dehydration. A rectal swab was taken for bacterial culture and a faecal sample was collected whenever possible (in 89% patients) for microscopical examination. Information was recorded on the duration of stay in hospital and on the treatment given.

Laboratory methods

To culture *Shigella* spp, a rectal swab from each of the 2,635 patients was plated directly onto Salmonella-Shigella agar and MacConkey's agar media. To culture *Campylobacter,* every alternate swab was also plated onto Campy-BAP agar and the plates were incubated at 42°C for 48 hours. The species of *Shigella* and *Campylobacter* isolated were identified using standard methods (3-5).

All the isolates of *Shigella* spp. were tested for their sensitivity to tetracycline, ampicillin, co-trimoxazole, chloramphenicol, streptomycin, furazolidone, erythromycin, kanamycin, and gentamicin using the methods of Bauer *et al.* (6).

Stool samples were obtained for microscopical examination from only 2,343 patients (89%); the remainder were unable to provide samples. The pH of fresh stools was tested using litmus paper, and a direct smear was examined microscopically for red blood corpuscles, white blood cells, and for the cysts and trophozoites of *E. histolytica* (7). The number of cells seen was quantified approximately by counting the number seen in a high power field (x 400).

Statistical tests

The results were analysed by Chi-squared statistics.

Results

Age and sex of patients sampled

Of the 2,635 patients sampled, 9.1% were less than 1 year of age, 31.3% between 1 and 4 years, 20.4% between 5 to 14 years and the remaining 39.2% were aged 15 years or above. More than half (56.4%) of the patients were male.

Microbiological culture

Shigella spp were isolated from the faeces of 292 patients (11.1%). Of these isolates, 181 were *Shigella dysenteriae* type 1 (62%), 79 were *S. flexneri* (27.1%), 17 were *S. boydii* (5.8%), 10 were *S. sonnei* (3.4%) and 5 were *S. dysenteriae* type 2 (1.7%). *Campylobacter jejuni* was the only species of *Campylobacter* identified and was isolated from 148 (11.2%) of the 1,318 samples tested.

Age-specific infection rates

Shigella spp were isolated most frequently from children aged 1-2 years (154/1000), and in patients aged 60 years or more (147/1000), and least frequently in children under one year of age (58/1000). In contrast, *S. dysenteriae* type 1, the predominantly isolated species, was isolated most frequently in patients aged 5-9 years (94/1000) and 10-14 years (116/1000) (Table I) and more frequently from male patients (84/1000) than from females (49/1000; $X^2=13.0$; $p<0.001$).

TABLE I — AGE DISTRIBUTION OF PATIENTS INFECTED WITH *SHIGELLA* SSP

Age in (years)	No. of patients studied	*Shigella* species (isolations/1000 patients)					
		S. flexneri (n=79)	*S. dysenteriae* type 1 (n=181)	*S. sonnei* (n=10)	*S. dysenteriae* type 2 (n=5)	*S. boydii* (n=17)	Total (n=292)
<1	240	21	29	0	4	4	58
1-2	520	54	75	15	0	10	154
3-4	306	56	78	0	0	3	137
5-9	339	12	94	6	3	6	121
10-14	198	10	116	0	0	5	131
15-29	503	18	54	0	2	2	76
30-44	283	18	46	0	0	7	71
45-59	151	26	66	0	7	13	113
60+	95	53	63	0	11	21	147
Total	2,635	30	69	4	2	6	111

Seasonality of shigellosis

Two peaks of shigellosis was observed - one during monsoon (June-September) caused by *S. dysenteriae* type 1, and the other in winter (December) caused by *S. flexneri*. The lowest isolation rate of *Shigella* spp was in February, when the admission rate of diarrhoea patients in the Matlab hospital was also at its lowest level.

Stool examination

The results of the stool examinations are shown in Table II. Blood was visible to the naked eye in 68% of the faeces positive for *Shigella* spp on culture, but in only 18% of samples showing *E. histolytica*, in 18% of samples yielding *C. jejuni* on culture and 12% of samples from the ramaining patients. Mucus visible to the naked eye was seen in similar proportions of stool samples from patients proved to be infected with *Shigella* spp (88%) and *E. histolytica* (84%) but was less common in patients with *C. jejuni* (51%) and in patients with diarrhoea not due to shigellosis, (36%). Stool samples positive for *Shigella* on culture were more often alkaline than those from patients yielding other pathogens (251/276 vs 1580/2052; $X^2=28.3$; $p<0.001$).

The results of the stool examinations from patients infected with different species of *Shigella* are shown in Table III. Blood was visible to the naked eye in 68% of all samples from which *Shigella* was isolated and in 87% of samples from patients infected with *S. dysenteriae* type 1.

Shigellosis: clinical characteristics, treatment and outcome

The results of the clinical characteristics of the patients with shigellosis are shown in comparison with subjects not found to be infected with *Shigella* spp. in Table IV. A large proportion of the patients with shigellosis presented with bloody stools (65%). Patients infected with *S. dysenteriae* type 1 were more likely to have bloody stools than patients infected with other species of *Shigella* (150/181 vs 40/111; $X^2=77.8$; $p<0.001$).

Patients with shigellosis less frequently presented with moderate-to-severe dehydration (53/292 vs 1182/2343; $X^2=108.9$; $p<0.0001$), but required antibiotics more often (245/292 vs 1429/2343; $X^2=58.8$; $p<0.0001$). Patients infected with *S. dysenteriae* type 1 required antibiotics more frequently than those infected with other species of *Shigella* (166/181 vs 79/111; $X^2=21.5$; $p<0.001$).

Sensitivity to antibiotics of Shigella spp

The results of the tests of resistance to

TABLE II — RESULTS OF NAKED EYE AND MICROSCOPICAL EXAMINATIONS OF FAECES FROM PATIENTS INFECTED WITH *SHIGELLA* SPP, *CAMPYLOBACTER JEJUNI* AND *ENTAMOEBA HISTOLYTICA* COMPARED WITH THE REMAINDER. (DATA IN % OF PATIENTS)

Examination	*Shigella* spp (n=276)	*Campylobacter jejuni* (n=130)	*Entamoeba histolytica* (n=44)	All other patients (n=2,052)
Naked eye				
Blood	68	18*	18*	12*
Mucus	88	51*	84	36*
Microscopic				
RBCs †				
0	24	65*	43*	59*
1-10	15	18*	31*	18*
>10	61	18*	25*	23*
WBCs †				
0	24	62*	18*	61*
1-10	13	22*	75*	19*
>10	63	15*	7*	20*
Macrophages †				
0	34	81*	89*	78*
1-5	34	15*	11*	14*
>5	32	4*	0*	8*
pH				
Acidic	9	31*	14	33*
Alkaline	91	69*	86	77*

* Value is significantly different ($p<0.01$ by Chi-square test) from value for patients infected with *Shigella*.
† Number of cells per high power field (magnification × 400).

antibiotics on 292 isolates of *Shigella* are presented in Table V and show that 11% were resistant to ampicillin and 61% to co-trimoxazole. Most of the resistance to co-trimoxazole ocurred among isolates of *S. dysenteriae* type 1 (97%), and there was little resistance among other species or strains (2%). Resistance to chloramphenicol, tetracycline, streptomycin, furazolidone, and erythromycin was common.

Discussion

In comparison with earlier reports from the same hospital (8,9), there are some interesting differences. The first is the increased isolation rate of *Shigella*. This cannot be explained by improved diagnostic methods as they have remained the same, but one explanation may lie in the fact that before the present study was undertaken the hospital in Matlab was perceived by the community as a place to be treated for watery diarrhoea rather than for dysentery (10).

According to an earlier report (8) from Matlab (1978-1980), *S. flexneri* was the predominant species isolated (44/1000 patients), followed by *S. dysenteriae* type 1 (12/1000). In our study, the rate of isolation of *S. dysenteriae* type 1 (69/1000) far exceeded that of *S. flexneri* (30/1000)

TABLE III — RESULTS OF NAKED EYE AND MICROSCOPICAL EXAMINATIONS OF FAECES FROM PATIENTS INFECTED WITH *SHIGELLA* SPP, COMPARED BY SEROTYPE (DATA IN % OF PATIENTS)

Examination	*S.dysenteriae* type 1 (n=173)	*S.flexneri* (n=75)	*S.boydii* (n=16)	*S.sonnei* (n=7)	*S.dysenteriae* type 2 (n=5)	Total *Shigella* spp (n=276)
Naked eye						
Blood	87	37	25	43	40	68
Mucus	97	67	42	100	100	88
Microscopic						
RBC †						
0	14	37	56	57	20	24
1-10	15	16	6	29	0	15
>10	71	47	38	14	80	61
WBC †						
0	14	39	56	43	20	24
1-10	9	20	6	43	20	13
>10	77	41	38	14	60	63
Macrophages †						
0	21	56	56	71	20	34
1-5	43	19	13	14	40	34
>5	35	25	31	14	40	32
pH						
Acidic	5	15	19	14	0	9
Alkaline	95	85	81	86	100	91

† Number of cells per high power field (magnification × 400)

(X^2=42.09; $p<0.0001$). The difference between the ratios of isolations of *S. flexneri* to *S. dysenteriae* type 1 in these two periods was also highly significant (X^2=204.38; $p<0.0001$). These comparisons implicate that an epidemic caused by *S. dysenteriae* type 1 was possibly occurring during the study period.

Between 1978 and 1980, *Shigella* were isolated most frequently in the dry season after monsoon (September-December), but in our study there were two peaks of shigellosis - one during monsoon (June-September) due to *S. dysenteriae* type 1 and the other in winter (December) due to *S. flexneri*. This different seasonality of *Shigella* isolations is difficult to explain. The emergence of *S. dysenteriae* type 1 as the dominant species may be due to environmental factors favouring the transmission of the bacteria and/or an increased infectivity of the organism itself (11).

Blood visible to the naked eye was strongly suggestive of shigellosis: the specificity of a diagnosis made on this criterion was 87% and the sensitivity was 65%. The microscopical examination of faeces did not improve the sensitivity or specificity of the diagnosis. This finding underscores the importance of a naked eye

TABLE IV — CLINICAL CHARACTERISTIC AND TREATMENT OF PATIENTS INFECTED WITH VARIOUS *SHIGELLA* SPECIES AND THEIR COMPARISON WITH THOSE IN PATIENTS OF OTHER TYPES OF DIARRHOEA. (DATA IN % OF PATIENTS)

Clinical haracteristics	*Shigella species*						
	flex (n=79)	*dys.1* (n=181)	*sonnei* (n=10)	*boydii* (n=17)	*dys.2* (n=5)	total (n=292)	other diarrhoeas (n=2,343)
Signs and symptoms							
stool mucous	67	93	70	59	100	83	36
stool blood	39	83	30	24	40	65	13
abdominal pain	63	89	50	59	60	78	39
watery stool	56	23	60	76	40	36	81
vomiting	67	41	50	65	60	50	82
Physical signs							
temperature							
up to 100° F	91	74	90	65	60	78	90
> 100° F	9	26	10	35	40	22	10
dehydration							
none	36	54	60	18	40	46	21
mild	30	38	20	356	20	35	29
moderate	19	5	0	18	20	10	22
severe	14	3	20	29	20	9	29
Treatment							
ORS*	46	30	20	41	40	35	58
IVF †	25	13	20	41	20	20	36
antibiotics	70	92	30	65	80	84	61
Duration of stay (hrs.)							
< 72	87	82	100	88	100	85	92
72+	13	18	0	12	0	15	8
Case-fatality rate	0	2.2	0	0	0	1.4	0.4

Note : Comparison of all patients infected with *Shigella* all all patients infected with a species other than *Shigella* were significant at the $p<0.01$ level by Chi-square statistics for all clinical characteristics except case fatality rate which was significant at the $p<0.05$ level.

* ORS = Oral rehydration solution.

† IVF = Intravenous fluid.

examination of stool samples of diarrhoea patients by a physician or a health worker in rural area where laboratory facilities are not available. *C. jejuni* and *E. histolytica* were also detected, but as they both can be present in the faeces of people without diarrhoeal disease their significance as causes of dysentery in Matlab is uncertain.

Treatment with antibiotics has been shown to decrease the duration of shigellosis and to reduce the period that organisms are excreted (12). However, the rapid emergence of strains of *Shigella* resistant to antibiotics has made it difficult to choose a drug which is likely to be effective. For example, the results of the present study show

TABLE V — ANTIBIOTIC RESISTANCE PATTERN OF *SHIGELLA* ISOLATES

Stool Examination	Percentage of *Shigella* isolates resistant					
	S.dys.1 (n=181)	*S.flex* (n=79)	*S boydii* (n=10)	*S.sonnei* (n=17)	*S.dys.2* (n=5)	Total (n=292)
Tetracycline	98	89	60	41	60	90
Ampicillin	4	22	30	24	20	11
Co-trimoxazole	96	3	0	0	0	61
Streptomycin	98	86	50	47	40	89
Kanamycin	<1	4	0	0	0	1
Gentamycin	0	1	0	0	0	<1
Furazolidine	97	91	60	47	80	91
Erythromycin	98	92	50	71	60	92
Chloramphenicol	96	63	30	24	20	79

that the majority of isolates of *Shigella* were resistant to co-trimoxazole (61%) and that almost all isolates of *S. dysenteriae* type 1 were resistant (97%). Resistance to ampicillin was also found.

There are probably two main causes of the development of multiple resistance to drugs. The first is the unrestricted and inappropriate use of antibiotics, often in doses and dosages too small to have an effect (13). The second lies in the fact that resistance may be plasmid-mediated and that plasmids can be transferred from non-pathogenic to pathogenic species of bacteria (14). This means that if shigellosis is to be treated with drugs that are likely to be effective, then information must be collected regularly, perhaps by systematic surveys, on current resistance to drugs.

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