

Changing species distribution and antimicrobial susceptibility pattern of *Shigella* over a 29-year period (1980–2008)

F. KHATUN^{1*}, A. S. G. FARUQUE¹, J. L. KOECK^{2,3}, P. OLLIARO⁴, P. MILLET²,
N. PARIS⁵, M. A. MALEK¹, M. A. SALAM¹ AND S. LUBY¹

¹ International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh

² Centre René Labusquière, Université Victor Segalen Bordeaux 2, Bordeaux, France

³ Service de Biologie Clinique, Hôpital Militaire Robert Piqué, Bordeaux, France

⁴ UNICEF/UNDP/WB/WHO Special Programme for Research & Training in Tropical Diseases (TDR), Geneva, Switzerland

⁵ Société OPTIMA, Merignac, France

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SUMMARY

We studied changes in species distribution and antimicrobial resistance patterns of *Shigella* during 1980–2008, using the Diarrhoeal Diseases Surveillance system of Dhaka Hospital of ICDDR,B. In hospitalized patients *Shigella* prevalence decreased steadily from 8–12% in the 1980s to 3% in 2008. Endemic *S. flexneri* was the most commonly isolated species (54%). Epidemic *S. dysenteriae* type 1 had two peaks in 1984 and 1993, but was not found after 2000, except for one case in 2004. The therapeutic options are now limited: in 2008 a total of 33% of *S. flexneri* were resistant to ciprofloxacin and 57% to mecillinam. In the <5 years age group, severely underweight, wasted and stunted children were more at risk of shigellosis compared to well-nourished children ($P < 0.001$). Although hospitalization for *Shigella* diarrhoea is decreasing, the high levels of antimicrobial resistance and increased susceptibility of malnourished children continue to pose an ongoing risk.

Key words: Antimicrobial resistance, Bangladesh, diarrhoea, severe malnutrition, *Shigella*.

INTRODUCTION

Of the estimated 165 million annual episodes of shigellosis worldwide, 99% occur in low-income countries, largely in children aged <5 years, who represent 69% of the episodes and 60% of deaths [1]. A population-based study conducted in one urban slum of Bangladesh observed an incidence of shigellosis of 7.9/1000 person-years, but the rate was

60% higher (13.2/1000 person years) in children aged <5 years [2]. Childhood malnutrition is a major public health problem in Bangladesh, where >40% of pre-schoolchildren are malnourished [3]; these children are not only at higher risk of *Shigella* infections, but also experience complications and fatal outcomes more often [4, 5]. Disease severity and outcome differ substantially for the four species of *Shigella* (*S. dysenteriae*, *S. flexneri*, *S. boydii*, *S. sonnei*): *S. dysenteriae* type 1 (Sd1) outbreaks or epidemics are associated with higher rates of complications and deaths. In Bangladesh the majority of deaths due to endemic shigellosis were associated with *S. flexneri* [5, 6].

* Author for correspondence: F. Khatun, MBBS, M.Sc., Public Health Sciences Division, ICDDR,B, 68 Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka-1212, Bangladesh.
(Email: kfatema@icddr.org)

Distribution of serotype differs between industrialized and developing countries. Serotype distribution changed in Thailand as the country's economy developed [7]. Heterogeneous distribution of *Shigella* spp. and serotype have been observed in surveillance conducted in six Asian countries [2].

In Bangladesh, from 1968 to 1979, hospital-based studies conducted at urban Dhaka Hospital and rural Matlab Hospital of ICDDR,B observed that *S. flexneri* was the most predominant strain, with one Sd1 epidemic in 1973. The first documented outbreak of Sd1 in Bangladesh occurred in St Martin's Island, a coral island in the Bay of Bengal, from May to July 1973. Of 434 recognized cases 28 (6.4%) died, mostly infants and elderly people [8]. At that time, all strains of *Shigella* were found to be susceptible to most of the conventional antimicrobials used in Bangladesh, including tetracycline and chloramphenicol.

The prevalence of different species of *Shigella* and their antimicrobial susceptibility change over time, making treatment difficult [9]. Therefore, regular monitoring of species distribution and antimicrobial susceptibility is important, both for case management of individual patients, especially those at higher risk such as severely malnourished children aged <5 years, and for epidemic preparedness.

In this paper we aimed to describe the changing trend and antimicrobial susceptibility pattern of shigellosis by analysing the database of the Diarrhoeal Disease Surveillance system of ICDDR,B from 1980 to 2008.

METHODS

The Dhaka Hospital of ICDDR,B is located in the capital city of Bangladesh, Dhaka, and it provides care and treatment to about 100 000 patients each year, mostly residents of the city and its peri-urban areas, while a smaller proportion come from outside the city and even from distant parts of the country. The hospital has maintained a Diarrhoeal Disease Surveillance system since late 1979, enrolling a systematic 2% sample of all patients attending this facility. The system collects information on the clinical, epidemiological and demographic characteristics of every 50th patient, irrespective of age, sex or socio-economic status. In determining this sample frame, it was assumed that the system should be able to detect a significant difference ($P < 0.05$, two-sided) in the isolation of specific enteropathogens when cases were compared with controls (e.g. isolation rate of

5.8% in cases and 2.5% in controls), requiring an annual sample size of 660 cases and 660 controls. To facilitate subgroup analysis it was estimated that a sample size of 2000 would be adequate. A systematic sample of 2% would provide at least 2000 patients from a total of over 100 000 patients attending the hospital.

Trained research assistants interviewed patients, or the guardian of minors, using a structured questionnaire. A physician performed physical examinations at the time of hospitalization and recorded the findings. For this analysis we extracted data from January 1980 to December 2008, and reviewed patients with culture-positive *Shigella* infections.

For laboratory confirmation of *Shigella*, freshly collected stool specimens were immediately sent to the laboratory where they were inoculated onto MacConkey (MAC) and *Salmonella-Shigella* (SS) agar media plates. *Shigella* spp. were isolated and identified using standard laboratory methods [10], and the species and their serotype were confirmed using commercial antisera kits (Denka Seiken, Japan) specific for polyvalent and monovalent antigens for all serovars. The same culture method was used during the entire study period. Antimicrobial susceptibility of the isolated strains was determined by the disc diffusion method as recommended by the Clinical Laboratory and Standard Institute, CLSI [formerly the National Committee for Clinical Laboratory Standards (NCCLS)] [11]. The antibiotic discs used in this study (Oxoid, UK) included ampicillin (10 mg), mecillinam (25 mg), nalidixic acid (30 mg), trimethoprim-sulphamethoxazole (T-S) (25 mg), and ciprofloxacin (5 mg). *Escherichia coli* ATCC 25922 was used as the control strain for susceptibility studies [11].

χ^2 was used for assessing trends, and $P \leq 0.05$ considered as significant. For children aged <5 years, we analysed the risk of *Shigella* infection between malnourished and well-nourished children by calculating risk ratios (RR) and their 95% confidence intervals (CI).

Severe malnutrition was defined as (all values are z scores): height-for-age < -3 ; weight-for-age < -3 , and weight-for-height < -3 ; using the World Health Organization reference value [12]. Moderate malnutrition was defined as: height-for-age -3 to > -2 , weight-for-age -3 to > -2 , and weight-for-height -3 to > -2 . We considered children as well nourished if their weight-for-age, height-for-age and weight-for-height were -2 or more.

Table 1. Proportion of *Shigella* species distribution 1980–2008, Dhaka Hospital Surveillance system, ICDDR,B

Species	No. of isolates	Proportion (%) of <i>Shigella</i> spp., median (IQR)
<i>Shigella flexneri</i>	4157	55 (46–63)
<i>Shigella dysenteriae</i> 1	1457	17 (0–25)
<i>Shigella boydii</i>	1002	14 (10–21)
<i>Shigella sonnei</i>	458	7 (4–9)
<i>Shigella</i> spp.	501	4 (2–6)

IQR, Interquartile range.

RESULTS

In total, 83 073 patients were enrolled in the Diarrhoeal Disease Surveillance system at the Dhaka Hospital of ICDDR,B between 1980 and 2008. The stool/rectal swab cultures of 7575 (9·1%) were positive for *Shigella*. Of all patients infected with *Shigella*, 3648 (49%) were aged <5 years, 961 (13%) were aged 5–14 years, and 2891 (39%) were aged ≥15 years. The age distribution remained stable over time.

Of all *Shigella* strains, 55% were *S. flexneri*, 19% were *S. dysenteriae*, and 13% *S. boydii* (Table 1). The overall yearly isolation rates of *Shigella* ranged between 8% and 12% during 1980–1995 (Fig. 1) and then gradually decreased to 3% in 2008 (χ^2 for trend $P < 0\cdot001$), except for a single year peak of 8% in 2000.

Two peaks of isolation of Sd1 occurred in 1984 and 1993 (Fig. 2), and then the isolation gradually declined. This serotype was not isolated after 2000, except for a single patient in 2004.

S. flexneri remained the most prevalent strain during the entire period of observation, except during the peak of Sd1 epidemics in 1984 and 1993 when the proportion of isolation of *S. flexneri* and *S. dysenteriae* were almost identical (Fig. 2). In both these epidemics, the strains of Sd1 had acquired resistance to newer antimicrobials, including tetracycline, T-S, and ampicillin in 1984, and nalidixic acid in 1993. In contrast, the development of resistance of *S. flexneri* to ampicillin, and T-S has been much slower. Similarly, resistance to nalidixic acid from 1986 to 1997 was a slow process, followed by a sharp rise from 1998 and reaching 60% in 2002. Since 2007 *S. flexneri* has begun to show resistance to ciprofloxacin and

mecillinam and by 2008, 33% of *S. flexneri* was resistant to ciprofloxacin and 56% to mecillinam. Other strains of *Shigella* such as *S. sonnei*, *S. boydii* and *Shigella* spp. together had a 12% resistance to mecillinam and 9% to ciprofloxacin in 2008.

Of *Shigella*-positive children aged <5 years, 9% were severely underweight, 10% severely wasted and 8% severely stunted, while only 5% of well-nourished children aged <5 years were *Shigella* positive. Compared to well-nourished children severely malnourished children were at greater risk of developing *Shigella* infection: severely underweight (RR 1·8, $P < 0\cdot001$); severe wasting (RR 2·1, $P < 0\cdot001$) and severe stunting (RR 1·7, $P < 0\cdot001$) (Table 2). The proportion of malnourished children aged <5 years with all aetiologies of diarrhoea has decreased over time from 14% in severely underweight in 1993 to 4% in 2008. Similarly stunting was 11% in 1993 and 4% in 2008 (data not shown).

DISCUSSION

At the Dhaka Hospital of ICDDR,B the proportions of patients admitted with shigellosis decreased over time from 12% in 1980 to 3% in 2008. A moderate reduction in the estimated number of *Shigella* infections and a large reduction of mortality have been reported by a literature review in Asia [13]. Similar reductions in developed countries have been attributed to improvements in safe water supply, adequate sanitation facilities, and maintenance of good personal hygiene [14]. We do not have a definitive explanation for the reduction in shigellosis in Bangladesh from our study, but higher rates of isolation of *Shigella* from severely malnourished children and the reductions in the proportions of severely malnourished children in Bangladesh in recent years, would suggest that improved nutrition could be a likely factor. The association between measles and shigellosis, including higher severity and deaths from these two conditions co-occurring is well documented; in Bangladesh the immunization coverage against measles was 70% in 1996 and 83% in 2007 [3, 15]. Vitamin A is an important factor in preventing infectious illnesses. The coverage of vitamin A capsule supplementation in children increased from 40% in 1995 to around 70% in 2003 [16]. An earlier study conducted at ICDDR,B demonstrated the therapeutic benefits of vitamin A supplementation when used as an adjunct to antimicrobial therapy [17]. Although vitamin A was used in case management of shigellosis,

Table 2. Nutritional risk factor for shigellosis in children aged <5 years from 1993 to 2008, Dhaka Hospital Surveillance system, ICDDR,B

	Number (%) of children aged <5 years in respective nutritional category (n=19 511)	Number (%) of children in this nutritional category with <i>Shigella</i> (n=1193)	Risk ratio* (95% CI)	P
Moderately underweight	5389 (27.6)	345 (6.4)	1.3 (1.2–1.5)	<0.001
Severely underweight	3566 (18.2)	312 (8.7)	1.8 (1.6–2.1)	<0.001
Moderate wasting	3231 (16.5)	261 (8.1)	1.7 (1.4–2.0)	<0.001
Severe wasting	712 (3.6)	71 (10.0)	2.1 (1.6–2.6)	<0.001
Moderate stunting	3878 (19.9)	252 (6.5)	1.4 (1.2–1.6)	<0.001
Severe stunting	2515 (12.9)	210 (8.3)	1.7 (1.5–2.0)	<0.001
Well nourished	8367 (42.9)	403 (4.8)	—	

* All groups are compared to the well-nourished group.

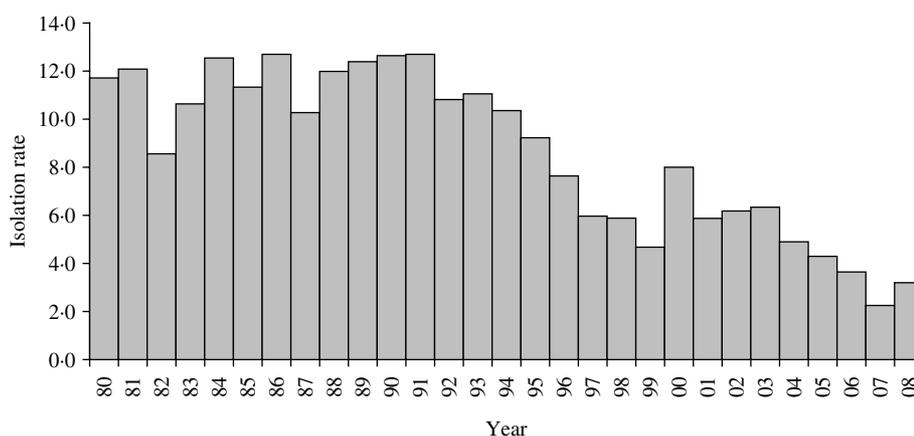


Fig. 1. Isolation rate (%) of *Shigella* between 1980 and 2008. Dhaka Hospital Surveillance system, ICDDR,B.

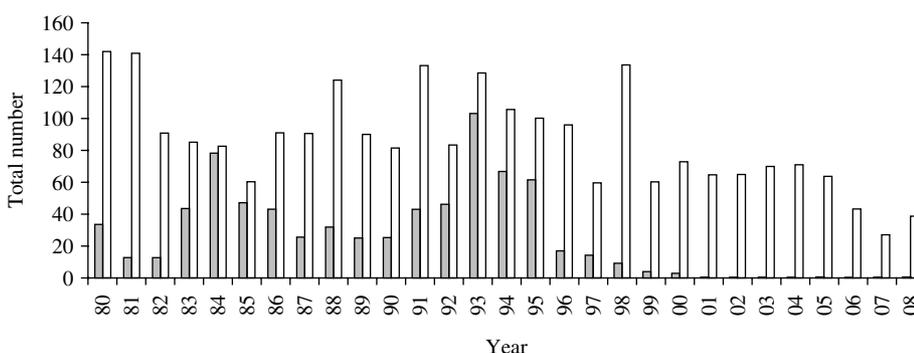


Fig. 2. Total numbers of *Shigella dysenteriae* type 1 (■) and *Shigella flexneri* (□), 1980–2008. Dhaka Hospital Surveillance system, ICDDR,B.

not as a preventive strategy, it seems reasonable to consider improved vitamin A status as a factor that might have contributed to reducing the prevalence of shigellosis in Bangladesh.

In Bangladesh, all four species of *Shigella* are present. *S. flexneri* is the most prevalent endemic species, and Sd1 has caused two epidemics since 1980 (in 1984 and 1993) but has not been isolated

since 2001 (Fig. 2). Hospital-based surveillance is not usually representative of an epidemic, yet in Bangladesh there were no population-based studies until 2001, so we considered the results of our hospital-based surveillance as a proxy indicator for these two epidemics [18, 19]. Both of these Sd1 epidemics were caused by strains that had acquired resistance to the antimicrobial agents in routine use for treating shigellosis. Of all the species of *Shigella*, the emergence of resistance and its quick progression is most notable in Sd1 strains.

After these two epidemics, separated by a decade-long interval, *Shigella* experts anticipated another epidemic in 2004 [20]. The factors underlying this trend of periodic epidemics have not been explored. Our data show that resistance to Sd1 is stable and has acquired resistance to additional antimicrobials, leading to a situation with limited options for empirical antimicrobial therapy. Despite fluctuations in the rates of isolation of Sd1, the serotype was isolated in all the years of our study period, except for 2001 to 2008, but in 2004 it was isolated from a single patient at Dhaka Hospital. The only other record was an outbreak of ciprofloxacin-resistant Sd1 in northeast Bangladesh associated with an 8% case-fatality rate in late 2003 [21]. The reason behind the apparent disappearance of Sd1 from Bangladesh is not known, but Sd1 has returned after long dormant intervals, with resistance to additional antimicrobials, in both eastern India and Malaysia [22, 23].

Although not as rapid as Sd1, the growing resistance since 2007 in *S. flexneri*, particularly against ciprofloxacin and mecillinam (the WHO recommended first- and second-line treatment of shigellosis), is also a matter of concern. Resistance to quinolones is associated with chromosomal mutation in *Shigella*, or transfer of resistance factors from other co-inhibiting microbes that are resistant to the fluoroquinolones [24, 25]. The development of resistance to nalidixic acid, an older quinolone, raised concerns that resistance would also emerge against ciprofloxacin. Our data demonstrated that to be the case. Second-line drugs are azithromycin and ceftriaxone. Azithromycin has been tested and proved to be effective in adults, and is less costly and easier to use compared to parenteral ceftriaxone [26]. Although not evaluated in the treatment of shigellosis in children, there is no reason to believe that this drug will not be effective in children.

The rate of childhood malnutrition remains high in Bangladesh, despite a reduction from 56% of

children aged <5 years in 1990 to 45% in 2005 [27]. Compared to better nourished children, the prevalence of *Shigella* in our study was higher in underweight, stunted, and wasted children aged <5 years, which is similar to earlier observations [5]. Of all diarrhoeal diseases, the negative nutritional impact is greater with shigellosis, due to presence of fever, greater anorexia, and the loss of enteric proteins, and has been associated with decreased linear growth rate in previous studies in Bangladesh [4, 28].

Our dataset is hospital based, which might not represent the population at large, with a possible bias towards children with more severe disease and those who fail outpatient treatment. Thus, the prevalence of antimicrobial resistance may be less in the community. Over time, the number of hospital admissions increased because the population of Dhaka city has grown and referral mechanism towards ICDDR,B Dhaka hospital has improved. We performed systematic sampling and presented the proportion of *Shigella* infection, thus increased hospital admission did not affect our results.

Urban populations in Bangladesh have better access to healthcare facilities, including those services received from public and private clinics/hospitals, general practitioners, which could have been a factor for our observation of reducing prevalence of shigellosis. The availability of highly potent drugs, such as ciprofloxacin, that are available over the counter could also have contributed [29]. The prevalence of shigellosis in Kamalapur, an urban field area of ICDDR,B is higher than that observed in our study with an isolation rate of 13% between 2002 and 2004 [2]. In addition, isolation rates of *Shigella* were higher in rural Matlab Hospital of ICDDR,B and another rural community-based study in Mirzapur (Dr A. S. G. Faruque, personal communication; Matlab and Mirzapur surveillance data). A nationwide *Shigella* surveillance could provide a more complete estimate of *Shigella* prevalence.

Shigellosis is one of the few diarrhoeal diseases for which the WHO recommends routine antimicrobial therapy, using an effective agent to reduce morbidity, complications and deaths. It seems that Sd1, the serotype of *Shigella* that causes the most severe illness with greater complications and deaths and is the most efficient in acquiring resistance to antimicrobials, has disappeared from Bangladesh, at least temporarily. However, the rapid emergence of resistance of other *Shigella* strains, especially *S. flexneri*, the most prevalent endemic species, is a matter of concern. The

recent resistance to ciprofloxacin and mecillinam has left only azithromycin and ceftriaxone as drugs with proven efficacy in the management of shigellosis. However, ceftriaxone is only available in parenteral formulation that requires hospitalization in health-care facilities. It is also expensive, making it inaccessible, particularly to families of severely malnourished children of low socioeconomic status. Severely malnourished children are not only at a greater risk of shigellosis, but are also at greater risk of related complications and deaths.

Our data highlight the importance of hospital-based surveillance in understanding the changing epidemiology of *Shigella* infections, including changes in antimicrobial susceptibility that would be helpful in the case management of shigellosis. Although the proportion of patients hospitalized for shigellosis has decreased over the last 28 years, the increase in antimicrobial resistance, the continued susceptibility of malnourished children to *Shigella*, and the history of prior Sd1 outbreaks means that *Shigella* represents an ongoing threat to public health in Bangladesh.

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DECLARATION OF INTEREST

None.

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