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Inside

Rapid emergence and spread of high-level ciprofloxacin resistance in *shigella* species in urban and rural Bangladesh: implication for therapy

We reviewed stool cultures from patients with diarrhoea in urban Dhaka and rural Matlab from 2005 to 2009 to evaluate the prevalence of *Shigella* and its antimicrobial susceptibility. Among 70,876 stool specimens from Dhaka, *Shigella* was isolated from 3,683 (5%) and among 8,924 stool specimens from Matlab, *Shigella* was isolated from 723 (8%). *Shigella flexneri* was the most common strain identified both in Dhaka (58%) and Matlab (76%). Among all isolated *Shigella* strains, 3% of strains were resistant to 5 drugs, 60% to 4 drugs and 28% to 3 drugs. The proportion of strains that are resistant to ciprofloxacin increased in Dhaka and Matlab from near 0% in 2005 to 25% in 2009. High levels of drug resistance limit the effectiveness of empiric therapy for shigellosis.

Shigella infection due to multidrug-resistant strains continues to be a major public health problem associated with significant morbidity,

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mortality, economic and health burden in developing countries, including Bangladesh. Of the four species, *S. boydii* and *S. sonnei* usually cause a milder and self-limited disease; while *S. flexneri* and *S. dysenteriae* are commonly isolated in endemic areas. Among *S. dysenteriae* strains, the *S. dysenteriae* type 1 (Sd1) causes severe disease with epidemic potential. Such outbreaks due to multidrug-resistant Sd1 occurred in Bangladesh and India in 1972-74, 1983-84 and 1993-94 (1-3). Antimicrobial therapy is recommended for patients to reduce symptoms and limit duration of fecal excretion of *Shigella* species and thereby its spread.

Low cost and easily available antibiotics such as sulphaguanidine, chloramphenicol, tetracycline, ampicillin and cotrimoxazole are no longer effective against *Shigella* species. Increasing resistance to nalidixic acid and mecillinam has been reported earlier (3). Since the late 1990s, the fluoroquinolones, including ciprofloxacin, norfloxacin, and ofloxacin, have been the drug of choice for multidrug resistant *Shigella* infections. Strains resistant to multiple drugs, including ciprofloxacin and norfloxacin, were reported from India and Nepal (4-6). Ciprofloxacin-resistant Sd1 cases were also reported from Bangladesh in 2003 from one location (7). Antimicrobial resistance of *Shigella* is now changing frequently in different geographic locations. It is thus important to review the current pattern of antimicrobial resistance of *Shigella* in Bangladesh. The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) monitored the isolation rate and antimicrobial resistance pattern of *Shigella* both in urban Dhaka and in rural Matlab. The isolation rate and the pattern of antimicrobial resistance of *Shigella* species from 2005 to 2009 is presented here.

We reviewed the results of stool cultures from fecal specimens from 79,800 diarrheal patients who were either hospitalized at Dhaka and Matlab hospitals, or referred cases from public or private hospitals and clinics in and around Dhaka city. All specimens were cultured onto Salmonella-Shigella agar (SSA), MacConkey agar (MCA) and Tellurite-taurocholate gelatin agar (TTGA) and incubated 16-18 hrs aerobically at 35-37°C. Non-lactose fermenting suspected *Shigella* colonies on agar plates were selected for identification biochemically and serologically following the standard method (8). Antimicrobial susceptibility was evaluated by disk diffusion following recommendations of the Clinical Laboratory Standard Institute [CLSI], (9) using commercially available antimicrobial disks of ampicillin (10ug), cotrimoxazole (25ug), nalidixic acid (30ug), mecillinam (25ug), and ciprofloxacin (5ug). (Oxoid, Basingstoke, UK). The inhibition zones were measured and interpreted as sensitive or resistant according to CLSI breakpoints. *E. coli* ATCC 25,922 was used as a control. The MIC of ciprofloxacin was determined by the E-TEST (AB Biodisk, Sweden). Multidrug resistance was defined as any species of *Shigella* that exhibited resistance to more than two antimicrobial agents.

Of the 70,876 stool specimens from Dhaka, from 2005 to 2009 *Shigella* was

isolated from 3,683 (5%). Among 8,924 stool specimens from Matlab, *Shigella* was isolated from 723 (8%). In Dhaka, *S. flexneri* (58%) was most frequently isolated followed by *S. boydii* (20%), and *S. sonnei* (13%) (Table 1). In Matlab, the majority of strains were *S. flexneri* (76%) followed by *S. boydii* (10%) and *S. sonnei* (7%).

Table 1: Distribution of Shigella *isolates in Dhaka and Matlab: 2005-2009*

Species name	Dhaka n (%)	Matlab n (%)	Total n (%)
S. dysenteriae	273 (7)	45 (6)	310 (7)
S. flexneri	2,127 (58)	552 (76)	2,679 (61)
S. boydii	718 (20)	73 (10)	791 (18)
S. sonnei	480 (13)	50 (7)	530 (12)
Non-typable	85 (2)	3 (1)	88 (2)
Total	3,683 (100)	723 (100)	4,406 (100)

The proportion of *Shigella* isolates from Dhaka that were resistant to ampicillin was 24%, to cotrimoxazole 71%, to nalidixic acid 75%, to mecillinam 18%, and to ciprofloxacin 11% (Table 2). *S. flexneri* was the species that was most commonly resistant to ciprofloxacin (16%). The proportion of *Shigella* isolates from Matlab that were resistant to ampicillin was 48%, to cotrimoxazole 61%, to nalidixic acid 74%, to mecillinam 10%, and to ciprofloxacin 10%. Again, *S. flexneri* was the species that was most commonly resistant to ciprofloxacin (12%). All ciprofloxacin resistant strains in Matlab and Dhaka had a minimum inhibitory concentration ≥32µg/ml. Among all isolated *Shigella* strains, 3% of strains were resistant to all 5 drugs, 60% to 4 drugs, and 28% to 3 drugs. Among patients with strains of multiple drug-resistant *Shigella*, 78% were children less than five years and 58% were male. for all *Shigella* species, the proportion of strains resistant to ciprofloxacin increased in Dhaka and Matlab from near 0% in 2005 to 25% in 2009 (Figure 1).

Reported by: Clinical Laboratory Services, Laboratory Sciences Division, ICDDR,B

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Comments

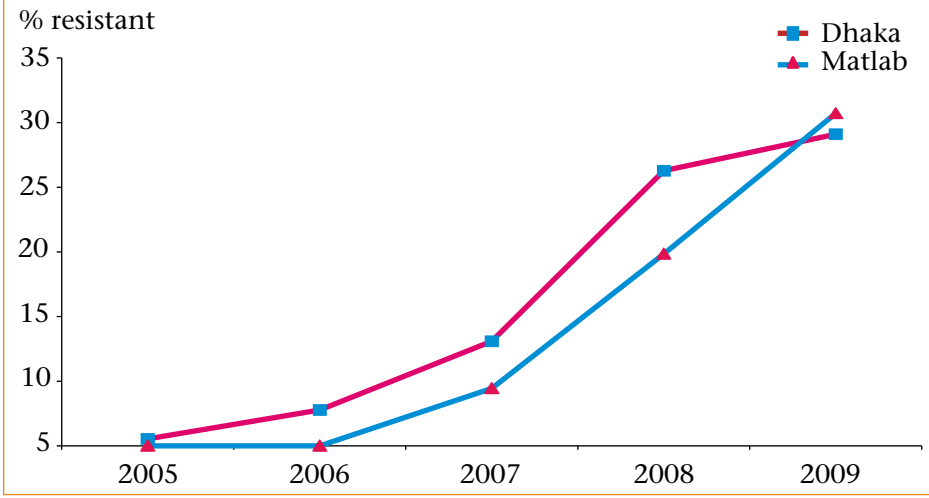
In the 1970s nearly all shigellae isolated in Bangladesh were susceptible to commonly used, low cost antimicrobials. Over the years, circulating *Shigella* strains have acquired increasing resistance to commonly affordable antimicrobials, including ampicillin, cotrimoxazole, nalidixic acid and mecillinam, leading to ciprofloxacin becoming the drug of choice to treat cases with bloody diarrhoea (3). Ciprofloxacin is considered superior to other

fluoroquinolones because of its bioavailability in higher concentration in the gut than other members.

Table 2 Antimicrobial resistance pattern of multidrug resistant Shigella *by species in Dhaka and Matlab: 2005-2009*

	Total strains	Ampi-cillin n (%)	Cotri-moxazole n (%)	Nalidixic Acid n (%)	Mecil-linam n (%)	Cipro-floxacin n (%)
Dhaka						
S. dysenteriae	273	43 (16)	194 (71)	148 (54)	17 (5)	4 (1)
S. flexneri	2,127	669 (31)	1,491(70)	1,732(81)	515 (24)	344 (16)
S. boydii	718	144 (20)	383 (53)	399 (56)	98 (14)	10 (1)
S. sonnei	480	20 (4)	469 (98)	420 (88)	19 (4)	29 (6)
Non typable	85	19 (22)	76 (89)	74 (87)	22 (26)	3 (4)
Total	**3,683**	**895 (24)**	**2,613(71)**	**2,773(75)**	**671 (18)**	**390 (11)**
Matlab						
S. dysenteriae & others	45	17 (38)	23 (51)	14 (31)	6 (13)	1 (2)
S. flexneri	552	298 (54)	340 (62)	451 (82)	62 (11)	67 (12)
S. boydii	73	26 (36)	38 (52)	29 (40)	1 (1)	0 (0)
S. sonnei	50	3 (6)	40 (80)	38 (76)	1 (2)	2 (4)
Non typable	3	3 (100)	2 (67)	3 (100)	0 (0)	2 (67)
Total	**723**	**347 (48)**	**443 (61)**	**535 (74)**	**70 (10)**	**72 (10)**

Figure 1:* Shigella *resistance to Ciprofloxacin : 2005-2009



Some strains of *Shigella* resistant to ciprofloxacin and other fluoroquinolones have been reported from India and Nepal (4-6). Sd1 was the first ciprofloxacin resistant strain of *Shigella* identified in Bangladesh in 2001 (7). The last

outbreak and/or epidemic caused by mecillinam resistant Sd1 was recorded in Bangladesh during 1993-94 (3). The previously observed 10-year cycle for Sd1 epidemics, and the appearance of ciprofloxacin resistant Sd1 isolates in May and June 2003 in Matlab and Dhaka, led to predictions that potential epidemics occurring in that period might acquire resistance to ciprofloxacin (10). However, we identified only 4 ciprofloxacin resistant Sd1 isolates from 2005-2009 (Table 2).

These current data show a high level of resistance to most commonly used antimicrobials among strains of *Shigella* and a dramatic increase in resistance to ciprofloxacin. Ciprofloxacin resistance is the most common among *S. flexneri*, but is seen across all species of *Shigella*. Presumably the widespread use of ciprofloxacin, that is available over-the-counter for treating human disease, as well as its widespread use in the agricultural sector, has conferred a survival advantage to strains of *Shigella* that have ciprofloxacin resistance. The higher proportion of ciprofloxacin resistant isolates in Dhaka may reflect wider use of the drug in an urban setting, compared to rural Matlab.

Surveillance for antimicrobial resistance among strains of *Shigella*, including vigilant assessment for the possibile emergence of ciprofloxacin-resistant strains of Sd1, should continue. The high prevalence of antimicrobial resistance among *Shigella* isolates noted in this study limits safe and efficacious treatment options for shigellosis, particularly for children.

This study highlights the need to develop newer classes of antibiotics to treat shigellosis cases that are orally effective against *Shigella* species and other highly resistant enteric pathogens. A concerted effort by drug producers is required for the development of new effective drugs in parallel with the efforts to develop an appropriate vaccine.

References

1. Rahaman MM, Khan MU, Aziz KMS, Islam MS, Kibriya AKMG. An outbreak of dysentery caused by *Shigella* dysenteriae type 1 on a coral island in the Bay of Bengal. *J Infect Dis* 1975;132:15-9.
2. Hossain MA, Albert MJ, Hasan HZ. Epidemiology of shigellosis in Teknaf, a coastal area of Bangladesh: a 10-year survey. *Epidemiol Infect* 1990;105:41-9.
3. Hossain MA, Rahman M, Ahmed QS, Malek MA, Sack RB, Albert MJ. Increasing frequency of mecillinam resistant *Shigella* isolates in urban Dhaka and rural Matlab, Bangladesh: a 6-year observation. *J Antimicrob Chemother* 1998;42:99-102.
4. Sur D, Niyogi SK, Sur S, Datta KK, Takeda Y, Nair GB, *et al*. Multidrug-resistant *Shigella* dysenteriae type 1: forerunners of a new epidemic strains in eastern India? *Emerg Infect Dis,* 2003;9:404-5.
5. ICDDR,B. Antimicrobial resistance surveillance for selected infectious

disease pathogens in Nepal, 1999-2003. Final report. 2003, 32-7 p.

6. Wilson G, Easow JM, Mukhopadhyay C, Shivananda PG. Isolation and antimicrobial susceptibility of *Shigella* from patients with acute gastroenteritis in western Nepal. *India J Med Res* 2006;123:145-50.
7. Naheed A, Kalluri P, Talukder KA, Faruque AS, Khatun F, Nair GB, *et al*. Fluoroquinolone-resistant *Shigella* dysenteriae type 1 in northeastern Bangladesh. *Lancet Infect Dis* 2004;4:607-8.
8. Ewing WH. Edwards and Ewing's identification of enterobacteriaceae, 4th edn. New York: Elsevier, 2004.
9. National Committee for Clinical Laboratory Standard Institute (CLSI), 2005. Performance standards for antimicrobial susceptibility testing. Fifteenth informational supplement M100-S15. 25 (1). CLSI, 2005; Wayne, PA.
10. ICDDR,B. Alert – Potential for epidemic dysentery in Bangladesh associated with the emergence of multidrug resistant *Shigella* dysenteriae type 1, resistant to ciprofloxacin. *Health Sci Bul* 2003;1:15-6.

Street dwellers' preference for health care services in Dhaka, Bangladesh

Street dwellers in Dhaka city have limited access to effective healthcare services and are vulnerable to poor health. There is no health service delivery mechanism targeting this marginalized group of people. This paper presents the findings of a formative research exploring ways healthcare services could be made more accessible to street dwellers in Bangladesh. The street dwellers requested health care facilities that could be available anytime they needed services and that respected their desire for privacy. Operations research based on these initial findings could help to develop an appropriate mechanism for providing healthcare services to this population.

Street dwellers in Dhaka, the capital city of Bangladesh, are people who sleep in public places, such as streets, railway stations, bus stations, parks, religious centres, construction sites and in cemeteries (1-3). They are among the most vulnerable people in an urban setting and morbidity among them is high (4-6). A previous study in 2008, focusing on the health needs and health care seeking behaviours of street dwellers, highlighted their vulnerability. It found that 72% (n=448) of female and 48% (n=448) of male street dwellers had symptoms of illness at the time of data collection. The most common general complaints included respiratory diseases, digestive problems, ear, eye and nose infections, severe head or chest pain, skin

infections, tuberculosis, and kidney infection (7). The female street dwellers were primarily affected by reproductive health problems, which included vaginal discharge, lower abdominal pain, and genital itching and burning. Almost all street dwellers reported their children less than five years of age had more than one symptom associated with acute respiratory infection in the two weeks prior to data collection. Additionally, 37% of female and 34% of male street dwellers reported that their children less than five years of age had diarrhoea in the two weeks prior to data collection.

About half of the female street dwellers and one-third of the male street dwellers did not seek healthcare services for their illness. Of those who did, more than half of the females and two-thirds of the males bought medicine from drug sellers at the nearest pharmacy (7). The street dwellers did not buy the full course of medicine from pharmacies due to lack of money. This suggests there is a lack of access to adequate healthcare services for this marginalized group of people.

To explore developing a service delivery mechanism to provide essential healthcare services to urban street dwellers in Dhaka, researchers from ICDDR,B conducted a qualitative formative research study from April to July 2009. This paper presents the findings that highlight ways to make healthcare services more accessible and attractive to this population.

We purposively selected two areas in Dhaka city. Kamlapur serves as the entry point for many rural people on arrival in the city, while Karwan Bazar is a concentrated area of low-income accommodation. We used global positioning system (GPS) to determine a two kilometre radius from a central point in both Kamlapur and Karwan Bazar to fix the two study site areas. Experienced qualitative researchers collected data through focus group discussions and in-depth interviews.

A total of four focus group discussions, two with females and two with males, were conducted with ever-married street-dwellers aged 15 years and above. Six participants were purposively selected for each focus group discussion who met the criteria of sleeping in the study area for the last two weeks and who had reported at least one health complaint. One moderator conducted each session using a structured guideline and a tape recorder to capture the discussions.

In each of the two study areas, a list of all health care providers was prepared, including drug sellers, paramedics from government clinics or from non-government organizations, and doctors and nurses from private health care facilities. A total of 39 (20 in Kawran Bazar and 19 in Kamlapur) health care providers were listed. We randomly selected 10 key informants from this list, and conducted the in-depth interviews using a structured guideline.

In both the focus group discussions and in the in-depth interviews, we elicited responses focused on street dwellers' health care needs, health care

seeking behaviours and perceptions about what service delivery mechanism would be most accessible and attractive. We performed content analysis of the focus group discussion and in-depth interview transcripts using guideline categories that we systematically recorded.

Half of the focus group discussion participants were between 15 and 30 years of age and the others were more than 30 years (Table 1). The occupations of the female street dwellers were mainly scavenging and/or selling of scrap and waste paper, and being a house maid. Male street dwellers' main occupations were begging, van and rickshaw pulling, and non-formal construction work. Six out of 12 female participants were living alone, while the others stayed with their husbands and children. Eight out of the 12 male participants were living alone. There were two to four members in the families of the male street dwellers and most of them had been living on the streets for 5-10 years.

Table 1: Characteristics of street dwellers who participated in the focus group discussions

Characteristics	Females (n= 12)	Males (n=12)
Age		
15-30	06	06
30+	06	06
Occupation		
Maid servant	05	00
Scavenging scrap	05	00
Begging	01	04
Housewife	01	00
Van puller	00	03
Rickshaw puller	00	01
Day labour	00	01
Construction labourer	00	03
Family status		
Live with family	06	04
Live without family	06	08
Number of family members		
<2 children	00	00
2-4 children	08	07
>4 children	04	05
Time spent as street dweller		
<5 years	05	01
5-10 years	07	10
10+ years	00	01

All the female participants in the focus group discussions said they wanted health services from any kind of low cost health facility that was near to where they were living because of their high mobility. One of the female informants said:

> "We work the whole day outside and in the evening we return to the place where we reside. Then we have to cook our food and we are tired. It is not possible to go out and travel a long distance for treatment for a medical problem. Furthermore, we have to arrange money for transportation costs and that is not possible for us".

The majority of female and male informants generally preferred government facilities because they were free or very low cost. However, all female informants were concerned about privacy in the healthcare centres. They stated that for some kind of services, such as antenatal care, postnatal care, and reproductive tract infection and/or sexually transmitted diseases (*gopan rog*) check-ups, privacy is essential. They suggested that privacy could be maintained through using some sort of screen. All of them suggested that female health care providers are essential. One female informant said:

> "Without a screen made of cloth (*parda*) medical care is not suitable for women. For the sake of diagnosing the sexually transmitted disease (*gopan rog*) we have to show our body to a female doctor. It needs '*parda*' so that the outsider can't see us".

Two male participants also said that there are some secret diseases which cannot be disclosed in public. If there is an extra room or private place in the clinics it would make it easier for them to discuss their problems with male doctors.

The majority of the female focus group discussions participants preferred to get health care service from paramedics. These women explained that they can have easy access to them and can tell their problems to them without any hesitation, because paramedics know their situation, listen to them, and are sympathetic. They said qualified doctors (MBBS) don't treat them in a respectful manner and charge too much money. However, two women did prefer to go to an MBBS doctor because they believed they could get proper treatment. Among the two male focus group discussions, most participants preferred paramedics to provide treatment.

In the in-depth interviews, almost all the health care providers stated that the street dwellers could not access their services due to lack of money, and work schedules that did not align with normal working hours. The pharmacists reported that although they are open at night in the two study sites, financial constraints prevented the street dwellers from obtaining either the correct drugs and/or the correct dosage. The pharmacists noted that their lack of medical knowledge prevented them from being able to recommend proper treatment.

All key informants suggested providing healthcare services near to street dweller areas. One idea put forward was to organize satellite clinics that provide health care services once or twice a week during evening hours. However, they noted that the street dwellers must be informed about this schedule well ahead of time. The majority also suggested that the experienced paramedics, who usually have better interpersonal communication with the street dwellers, should be the providers. However, aside from being close to their dwelling, both female and male informants preferred any health care facility that would be available anytime they needed services, not only on a limited basis.

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Comments

This formative research suggests that street dwellers cannot access conventional health care services due to the financial and time constraints linked to their livelihoods. In exploring how to make healthcare services more accessible to street dwellers, the specific requirements that they described were longer, more flexible opening hours, free or low cost services, and quality services provided by paramedics.

In Bangladesh, there are a few non-governmental organizations (NGOs) that provide health care services that meet these criteria for street dwellers. For example, Marie Stopes Clinic Society has been supporting mobile clinic services for street dwellers on a small scale (8). Another NGO, Aparajeyo Bangladesh, provides limited medical care through drop-in-centres. However, there are no comprehensive services, either by NGOs or by government, to ensure the health needs of street dwellers are addressed (7).

The existing service delivery system is not convenient for street dwellers because the usual service hours do not match their free time due to their work schedule. Although there are pharmacies where the street dwellers may buy medicine, the drug sellers are not medically trained, and are not able to provide quality advice or treatment. Consequently, their health needs remain largely unmet through existing healthcare providers (7).

The question is how the healthcare needs of this population can be met. This is a difficult problem, because the demands for healthcare services far exceeds the resources available to meet them, not only for this group, but for other impoverished groups (1).

The idea to promote satellite clinics might be an efficient way to increase their access to health services. However, our informants did not see this as the best solution, and were equally concerned about the cost of quality care. For both men and women this meant privacy during consultations and

treatment with same sex health care providers.

By providing convenient and low cost quality health care services to street dwellers, the access to health care services would increase, thus decrease morbidity among this population. Further operations research could explore these findings.

References

1. Islam N. Addressing the Urban Poverty Agenda in Bangladesh: Critical Issues and the 1995 Survey Findings. Dhaka: Asian Development Bank, 1999: 29.
2. Bangladesh. Bangladesh Bureau of Statistics. Census of slum areas and floating population, volume 1. Dhaka: Bangladesh Bureau of Statistics, Planning Division, Ministry of Planning, Government of Bangladesh 1999:42-7.
3. Bangladesh Bureau of Statistics (BBS). Population census 2001: preliminary report, Dhaka: Bangladesh Bureau of Statistics, Planning Division, Government of Bangladesh, 2001. 39 p.
4. Anam S, Kabir R, Rai P. Staying alive: urban poor in Bangladesh. Dhaka: United Nations Children's Fund, 1993. 88 p.
5. Kedar Maharanjan (Vida Volunteer to SEEP), 2008, Survey analysis of Amrao Manush Project Mirpur 1, A project funded by Concern Worldwide Bangladesh (unpublished document).
6. Islam N. Urban poor in Bangladesh. Dhaka: Centre for Urban Studies, 1996:17-25.
7. Uddin MJ, Koehlmoos TL, Ashraf A, Khan AI, Saha NC, Hossain M. Health needs and health-care-seeking behaviour of street-dwellers in Dhaka, Bangladesh. *Health Policy Plan* 2009;24:385-94.
8. Marie Stopes Clinic Society. Proposal for joint collaboration between Marie Stopes, Bangladesh and United Commercial Bank Limited for an Intervention Targeting The Homeless People (unpublished document), 2008.

Hepatitis E outbreak in Rajshahi City Corporation

In April 2010, a joint investigation team from IEDCR and ICDDR,B investigated an outbreak of jaundice in Rajshahi City Corporation. A total of 2,162 suspected jaundice cases were identified in 30 of the 35 wards. Fifty-nine percent (35/61) of serum samples from cases were IgM positive for hepatitis E. Faecal coliform bacteria detected from two piped water samples suggested that municipal drinking water was contaminated with faeces. Those drinking municipal tap water (OR 2.2; CI 1.0-4.8) and having no formal education (OR 3.2, CI 1.2-9.0) were more likely to suffer from jaundice. To prevent recurrent outbreaks of hepatitis E in Bangladesh the public water supply needs to be upgraded so that appropriately treated water is supplied through a well maintained delivery system.

Hepatitis E is the commonest cause of acute hepatitis among adults in Asia, the Middle East and Africa (1). Hepatitis E outbreaks are frequently associated with contamination of drinking water in South Asia (2). Hepatitis E in pregnancy can lead to increased maternal and neonatal mortality and morbidity including postpartum haemorrhage, preterm labour, still births and abortion (3, 4, 5). Moreover, the rising burden of chronic liver diseases from hepatitis B and hepatitis C may lead to significant mortality from acute decompensation in cirrhotic patients due to super infection with Hepatitis E (6).

A national newspaper first reported the occurrence of a jaundice outbreak in Rajshahi City on 4 March 2010. The Chief Medical Officer of Rajshahi City Corporation, along with the municipal health team investigated the outbreak. Later a joint team from the Institute of Epidemiology, Disease Control and Research (IEDCR) and ICDDR,B joined the local team and conducted descriptive, environmental and laboratory investigations to determine the burden, aetiology and to identify risk factors.

The local team defined a suspected case of jaundice as a person of any age reporting yellow colouration of eyes or skin with onset during January to April 2010. Using this definition, the municipal health workers identified and recorded a total of 2,162 suspected cases of jaundice through house-to-house visits. The joint investigation team collected preliminary information from this line list.

We, the joint investigation team, conducted unstructured interviews with a purposively selected sample of suspected cases and local health authority personnel to generate a hypothesis about potential sources of infection. Out

of 30 affected wards, we selected 10 with the highest numbers of suspected jaundice cases. Among the 10 ward commissioners interviewed, one reported four deaths from jaundice and/or complications, including one pregnant woman, two elderly men with diabetes and one adolescent male. No detailed verbal autopsies were conducted. Given the epidemiological findings, clinical features, laboratory findings, widespread distribution of suspected cases, and past experience of a similar outbreak that had occurred in a low-income urban community last year (7), we suspected the outbreak to be caused by hepatitis E.

We conducted an unmatched case-control study to investigate the association between hepatitis E and potential risk factors, including municipal piped water supply in Rajshahi City. We defined a case of jaundice as a person of any age reporting yellow colouration of eyes or skin with onset from 22 March 2010 until the date of the interview who resided in any ward of Rajshahi City Corporation and whose symptoms were verified either by a health worker or supported by laboratory evidence, e.g. serum bilirubin. Among the 30 wards within Rajshahi City Corporation, we selected the 10 wards that had the highest numbers of suspected jaundice cases. Using our case definition, we identified 108 probable cases from the local team's line list of suspected cases. We then randomly selected six or seven cases (lottery method) from each ward to interview. We used a modified pretested questionnaire that had been used in a similar outbreak in an urban slum of Bangladesh (7) to compare drinking water sources and incidence of jaundice. We selected controls conveniently from friends, relatives, household members or neighbours of the selected cases who resided in the same ward and who had not reported yellowing of eyes or skin within the past six months until the date of interview. If a case could not be found, then the next case on the line list was approached for interview.

Assuming 50% of cases to be exposed to municipal supply drinking water compared to 30% of controls based on our preliminary investigation, we estimated a sample size of 135 (60 cases and 75 controls) to yield us 80% power to detect an association between supply water and jaundice if one truly existed. We selected 25% more controls to allow for prevalence of asymptomatic and past infections with hepatitis E, as they might not be at risk during the outbreak (8). We calculated Mantel-Haenszel common odds ratios (OR), 95% confidence intervals (CI) and significance levels (P).

We recruited 139 respondents (63 cases and 76 controls) for the case-control study and obtained informed oral consent before conducting interviews. Among these, 79 (57%) were males and 26% were students of Rajshahi University. The most common living arrangements were 4-5 households in a compound having a common stove, water source and toilet (41%). Most of the university students interviewed lived in hostels with a range of 18-44 people sharing a common stove, water source and toilet (Table 1).

Table 1: Demographic characteristics of respondents with reported jaundice (cases) and respondents without reported jaundice within the last six months (controls)

Demographic characteristics	Case (N=63)	Control (N=76)
Age in years		
Mean	28	28
Median (Range)	27 (2-65)	29 (3-65)
Education in completed years of schooling		
Mean	6.4	9.1
Median (Range)	6 (0-18)	10 (0-8)
Sex		
Male (%)	54.0	59.0
Female (%)	46.0	41.0
Average household members sharing the same stove		
Mean	5.0	6.9
Median (Range)	4 (2-22)	5 (1-44)
Average monthly expenditure*		
Mean	6,506	7,789.5
Median (Range)	5,000 (1,200-20,000)	6,000 (2,000-27,000)

*Bangladesh Taka (Tk. 69=US$ 1)

We collected 3ml of blood from every participant to test for IgM. We assumed approximately 10% of controls to have asymptomatic infections with HEV IgM antibodies in serum (8). The blood samples were tested in the IEDCR laboratory using HEV IgM ELISA 3.0 (MP Diagnostics) which demonstrated good sensitivity (88%) and excellent specificity (99.5%) in a previous study (9). Out of 63 cases, 61 samples were collected. Two refused to give blood. Samples were collected from all 76 controls. Among the 137 samples tested, 59 % (36/61) of serum samples from cases and 9% (7/76) samples from controls were IgM positive for HEV.

Cases of hepatitis E were more likely than controls to drink municipal piped water (OR-2.2; CI 1.0-4.8; P=0.05) and to lack formal education (OR 3.2; CI 1.2-9.0; P=0.02) (Table 2). Twenty-one (47%) cases and 37 (54%) controls had at least one household member affected by jaundice within the last one month. There was significant disruption of daily activities due to illness as 28 (62%) of the cases remained absent from work or school for an average of 14 days (Range = 2-30 days).

Table 2: Results of case-control analysis of risk factors for outbreak-associated hepatitis E, Rajshahi, 2010

Risk factors	Proportion of cases (N=45)	Proportion of controls (N=69)	Odds ratio (95% Confidence Interval)	P value
No education	12	7	3.2 (1.2-9.0)	0.02
Currently drinking supply water	22	21	2.2 (1.0-4.8)	0.05
Sharing toilet with a case outside of own household within last 6 months	18	26	1.1 (0.5-2.4)	0.80
Having another household member affected with jaundice (last two months)	21	37	0.8 (0.4-1.6)	0.50
Monthly household expenditure < 4000 taka	16	20	1.4 (0.6-3.0)	0.46
Foul smell in drinking water (last 1 month)	14	12	2.1 (0.9-5.2)	0.09
Colour change in drinking water (last 1 month)	16	19	1.5 (0.7-3.3)	0.37
Visible dirt in drinking water (last 1 month)	17	20	1.5 (0.7-3.3)	0.33
Drinking or eating outside home (last 1 month)	42	55	3.6 (1.0-13)	0.10
Having no sanitary / septic tank/advanced latrine	3	6	0.8 (0.8-3.2)	0.70

We conducted unstructured interviews with suspected cases, health workers and local leaders to collect information regarding the local water supply and sanitation. We also observed and explored the water distribution system in different wards of Rajshahi and identified several pathways for contamination of water. We collected water samples from three municipal distribution pumps and six shallow tube wells from areas with the highest concentration of cases and from five taps of households that had at least

three persons affected for bacteriological analysis using membrane filtration method. Water from shallow tube wells and pumps were devoid of faecal coliforms, but two tap water samples were contaminated with faecal coliforms (Table 3).

Table 3: Bacteriological test results of water samples

Source of sample	Number of faecal coliforms/c.c (Maximum allowable limit 0)
Pump water (N=3)	0
Shallow tube well (N= 6)	0
Pipeline water supply (N=6)	10-15 (Ward-16) 15-50 (Ward-18)

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Comments

Fifty-nine percent of serum samples from persons with reported jaundice were IgM antibody positive to hepatitis E virus. Cases of hepatitis E were more likely to drink tap water. Though water from shallow tube wells and municipal pumps were clean, water coming from several households' taps was contaminated with faecal coliforms. Thus epidemiologic, laboratory and environmental investigations suggested that the outbreak was caused by hepatitis E virus and contaminated drinking water was the probable source.

Several factors might have contributed to leakage in the pipelines and hence contamination of water in the distribution channels. During spring and summer the falling water table in Rajshahi leads to decreased water availability from shallow tube wells and an increased demand for water from the public water supply. Pipelines in some areas pass through poor quality surface water contaminated by urban effluent and even pass directly through open sewers. The intermittent water supply, usually unavailable 12 hours per day, results in negative pressure within the pipelines, which draws in contaminants that surround the pipes (10). The absence of faecal coliform organisms from pump water and the detection of faecal coliforms in two samples of the household drinking water suggest that such contamination occurs in the pipelines in Rajshahi.

This is the second large outbreak of hepatitis E within the last two years affecting urban communities in Bangladesh. The long period of disruption of daily activities due to such illness imposes substantial economic burden

to both the households and the community. Despite the high endemicity and burden in many low income countries, hepatitis E is seldom recognized as a public health priority in South Asia.

Maternal and neonatal deaths related to hepatitis E were not methodically looked for in this investigation. Systematically conducted house-to-house surveys to estimate maternal and neonatal mortality rates associated with epidemic transmission of hepatitis E, along with structured verbal autopsies and identification of risk factors for deaths, are needed in the future to better understand the burden of illness and identify possible interventions.

Preventing hepatitis E transmission requires the provision of safe drinking water through a well-maintained infrastructure along with regular testing of residual chlorine and coliform count from different distribution points. However, improving water and sanitary infrastructure is difficult to achieve in the short term in a resource poor setting. Boiling all drinking water, although an effective preventive strategy, may not be feasible throughout Rajshahi owing to lack of natural gas and high cooking fuel costs. Promotion of water treatment with chlorine at point-of-use could help to limit the extent of outbreaks in the short-term, and reduce the risk of waterborne diseases until more definitive steps can be implemented.

References

1. Emerson SU, Anderson D, Arankalle A, Meng XJ, Perdy M, Schlauder GG, *et al*. Hepevirus. Eighth Report of the International Committee on Taxonomy of Viruses. *Virus Taxonomy* 2004; 853-5.
2. Rab MA, Bile MK, Mubarik MM, Asghar H, Sami Z, Siddiqi S. Water-borne hepatitis E virus epidemic in Islamabad, Pakistan: a common source outbreak traced to the malfunction of a modern water treatment plant. *Am J Trop Med Hyg* 1997;57:151-7.
3. Hamid SS, Wasim JSM, Khan H, Shah H, Abbas Z, Fields H. Fulminant hepatic failure in pregnant women: acute fatty liver or acute viral hepatitis? *J Hepatol* 1996;25:20-7.
4. Hussain SH, Skidmore SJ, Richardson P, Sherratt LM, Cooper BT, O'Grady JG. Severe hepatitis E infection during pregnancy. *J Viral Hepat* 1997; 4: 51-4.
5. Patra S, Kumar A, Trivedi SS, Puri M and Sarin SK. Maternal and fetal outcomes in pregnant women with acute hepatitis E virus infection. *Ann Intern Med* 2007;147: 28-33.
6. Shata MT, Navaneethan U. The Mystery of Hepatitis E Seroprevalence in Developed Countries - Is There Subclinical Infection due to Hepatitis E Virus? *Clin Infect Dis* 2008;47:1032-4.
7. International Centre for Diarrhoeal Disease Research, Bangladesh. Outbreak of hepatitis E in a low income urban community in Bangladesh.

Health Sci Bul 2009;7:14-20.

8. Bryan JP, Tsarev SA, Iqbal M, Ticehurst J, Emerson S, Ahmed A, *et al*. Epidemic hepatitis E in Pakistan: patterns of serological response and evidence that antibody to hepatitis E virus protects against disease. *J. Infect Dis* 1994;1:517-21.
9. Legrand-Abravanel F, Thevenet I, Mansuy JM, Kamar N, Saune K, Vischi F, *et al*. *Clin Vaccine Immunol* 2009;15:772-4.
10. Clemette A, Amin MM, Ara S and Akan MMR. Background information for Rajshahi City, Bangladesh. WASPA Asia Project Report 2. 2006; [http://www.ngof.org/nrcreports/Asia%20Report-2.pdf, accessed on 12 September 2010].

Surveillance updates

With each issue of HSB, updates of surveillance data described in earlier issues are provided. These updated tables and figures represent the most recent observation period available at the time of publication. We hope these updates will be helpful to health professionals who are interested in current patterns of disease and drug resistance in Bangladesh.

Antimicrobial resistance patterns of 97 M. tuberculosis *isolates: September 2009-August 2010*

Drugs	Resistance type		Total n=97 (%)
	Primary n=83 (%)	Acquired* n=14 (%)	
Streptomycin	10 (12.0)	1 (7.1)	11 (11.3)
Isoniazid (INH)	6 (7.2)	2 (14.3)	8 (8.2)
Ethambutal	0 (0.0)	0 (0.0)	0 (0.0)
Rifampicin	0 (0.0).	0 (0.0)	0 (0.0)
MDR (INH+Rifampicin)	0 (0.0)	0 (0.0)	0 (0.0)
Any drugs	13 (15.7)	2 (14.3)	15 (15.5)

() column percentage *Antituberculous drugs received for 1 month or more

Antimicrobial susceptibility pattern of S. pneumoniae *among children <5 years during July-September 2010*

Antimicrobial agents	Total tested (n)	Susceptible n (%)	Reduced susceptibility n (%)	Resistant n (%)
Ampicilin	2	2 (100.0)	0 (0.0)	0 (0.0)
Cotrimoxazole	1	1 (100.0)	0 (0.0)	0 (0.0)
Chloramphenicol	2	2 (100.0)	0 (0.0)	0 (0.0)
Ceftriaxone	2	2 (100.0)	0 (0.0)	0 (0.0)
Ciprofloxacin	1	1 (100.0)	0 (0.0)	0 (0.0)
Gentamicin	2	0 (00.0)	0 (0.0)	2 (100.0)
Oxacillin	2	2 (100.0)	0 (0.0)	0 (0.0)

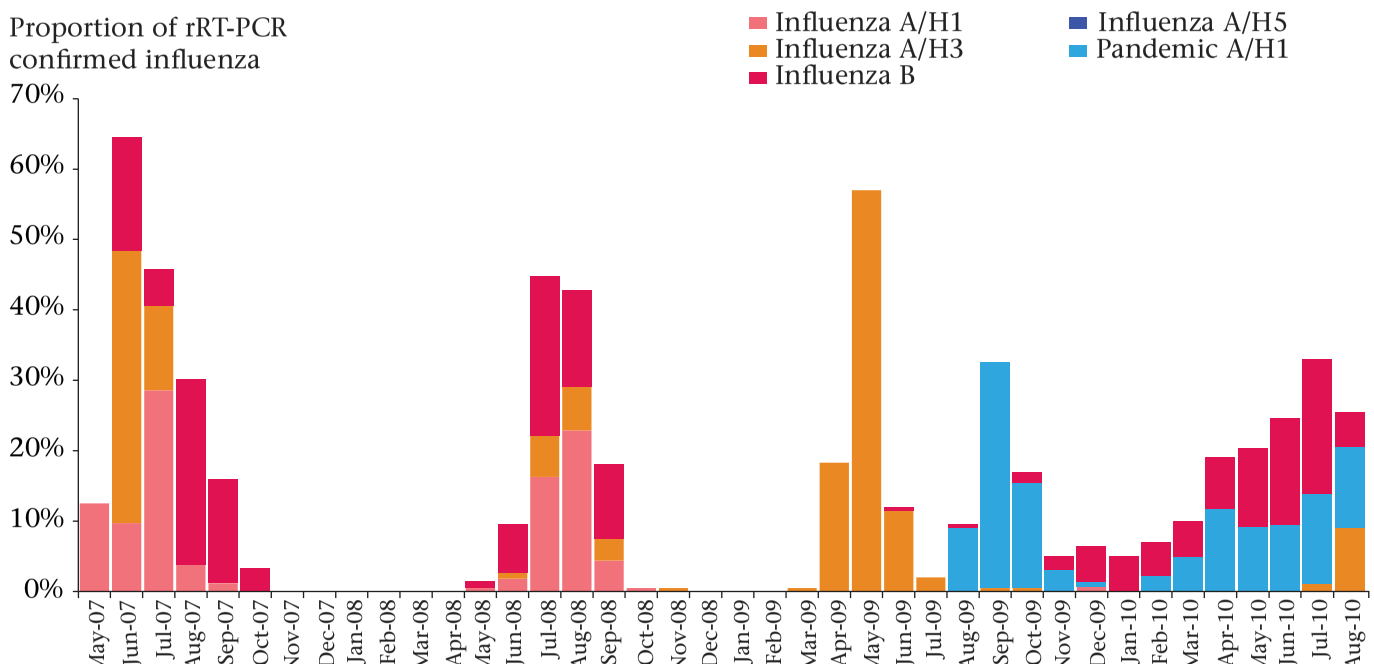
Source: ICDDR,B's urban surveillance in Kamalapur (Dhaka).

Antimicrobial susceptibility pattern of S. typhi *among children <5 years during January-March 2010*

Antimicrobial agents	Total tested (n)	Susceptible n (%)	Reduced susceptibility n (%)	Resistant n (%)
Ampicilin	17	8 (47.0)	0 (0.0)	9 (53.0)
Cotrimoxazole	17	10 (59.0)	0 (0.0)	7 (41.0)
Chloramphenicol	17	10 (59.0)	0 (0.0)	7 (41.0)
Ceftriaxone	17	17 (100.0)	0 (0.0)	0 (0.0)
Ciprofloxacin	17	0 (0.0)	17 (100.0)	0 (0.0)
Nalidixic Acid	17	0 (0.0)	0 (0.0)	17 (100.0)

Source: ICDDR,B's urban surveillance in Kamalapur (Dhaka).

Proportion of laboratory confirmed influenza among hospitalized severe acute respiratory illness (SARI) and outpatient influenza like illness (ILI) cases between May 2007 and August 2010



Source: Patients participating in hospital-based influenza surveillance in Dhaka National Medical College Hospital, Community-based Medical College Hospital (Mymensingh), Jahurul Islam Medical College Hospital (Kishoregonj), Rajshahi Medical College Hospital, Shaheed Ziaur Rahman Medical College Hospital (Bogra), LAMB Hospital (Dinajpur), Bangabandhu Memorial Hospital (Chittagong), Comilla Medical College Hospital, Khulna Medical College Hospital, Jessore General Hospital, Jalalabad Ragib-Rabeya Medical College Hospital (Sylhet) and Sher-e-Bangla Medical College Hospital (Barisal)

A street dwellers family in Dhaka City

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ICDDR,B
GPO Box 128, Dhaka 1000, Bangladesh
www.icddrb.org/hsb