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Inside

The role of the Biosafety Level 3 Laboratory in Bangladesh

Several infectious agents that circulate in Bangladesh, including HIV, *Bacillus anthracis*, highly pathogenic influenza virus, and multi-drug resistant *Mycobacterium tuberculosis*, cannot be studied safely in a typical laboratory with a low-level of biosafety. We report the construction and certification of a state of the art biosafety level 3 (BSL3) laboratory in Bangladesh to safely culture these important infectious agents for definitive diagnosis.

Laboratory diagnosis of infectious agents is a foundation of the prevention, containment, surveillance, and treatment of the associated illness. Several infectious agents, including novel pathogens, highly virulent viruses and multi-drug resistant pathogenic bacteria circulate in Bangladesh, but cannot be safely studied in laboratories with a low level of biosafety. Improving the ability of Bangladesh to respond to these threats requires containment laboratories within the country to safely diagnose, isolate and type these infectious agents.

Microorganisms are grouped considering their pathogenicity and risk they pose to humans and animals into four risk groups (RGs), from RG1 through RG4 (1,2). RG1 is non-pathogenic and thus

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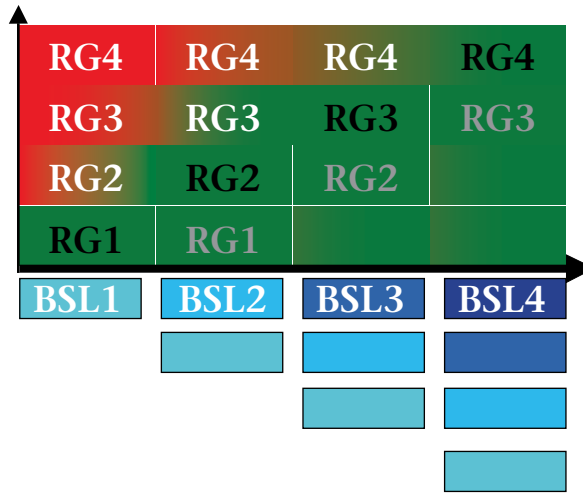
poses no risk to healthy individuals or animals. RG2 causes human or animal disease, but this is rarely severe. Effective treatment and preventative measures are available with a limited risk of spreading infection. RG3 pathogens cause serious human or animal disease, but do not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available. RG4 pathogens usually cause serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective prevention and treatment measures are not usually available for this group (1,2). To avoid exposure to these infectious agents, biohazard control and/or biosafety policies and procedures should be in place in research and diagnostic laboratories (1,2).

The difference in the infectious nature of various RGs of pathogens and the difference in their route of transmission requires adapting work practices, safety equipment, and facilities to minimize the exposure of workers and the environment to prevent transmission (1,2). Thus, biosafety levels (BSL) 1 through 4 have been developed with varying layers of protection depending on the RG of pathogens as shown in Figure 1 (1,2). Generally, BSL1, BSL2, BSL3, and BSL4 are suitable when working with agents belonging to RG1, RG2, RG3, and RG4, respectively. However, as a hazard increases, as shown in the red shadow in Figure 1 for each RG, then a combination of policies and procedures of the next level of BSL can be used to minimize the degree of hazard. Inversely, as shown by the grey shadow in Figure 1, the efficiency of laboratory diagnosis of infectious agents decreases with each higher level of BSL, due to more involved work

*Figure 1: Risk group (RG) of infectious agents and the need for the basic (BSL1 and BSL2) and containment (BSL3 and BSL4) laboratories for handling and manipulation of the agents**



*Layers of protection include: i) basic laboratory practices and precautions; ii) biohazard signage, BSC available for containment of infectious aerosol, autoclave available for waste treatment; iii) airborne precaution, containment precautions and a cascade negative pressure; and iv) negative pressure suit, and separate building.

practices and use of additional safety equipment and facilities.

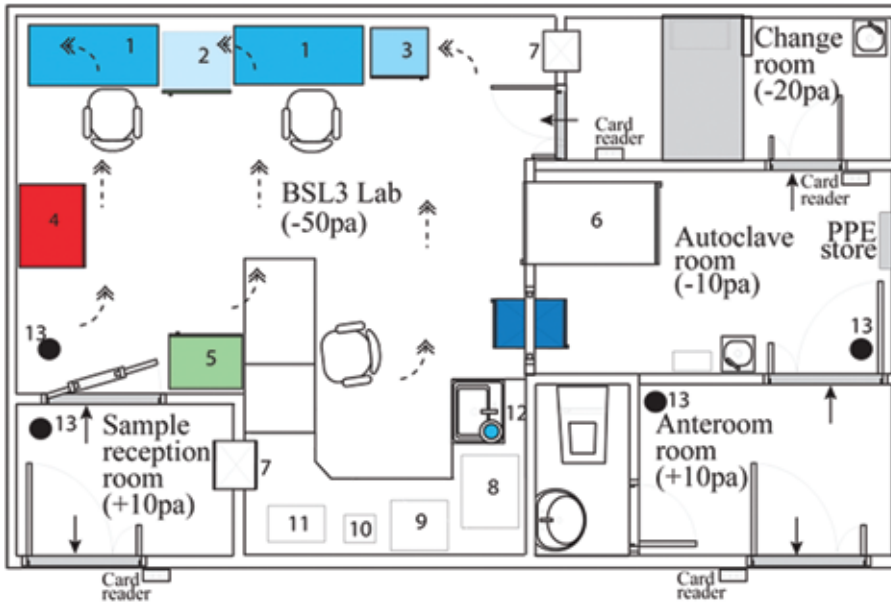
Because of the risk of infection to laboratory workers, all clinical laboratories are recommended to have policies and procedures that meet or exceed BSL2. Once an organism and its RG have been identified in a specimen, then further work practices and the appropriate BSL is determined for handling and manipulating the agent. The route of transmission is the major consideration for assigning BSL. Although handling and manipulation of RG2 agents can generally be performed in BSL2 (1-3), while working with a large volume of cultures or high number of agents with low infectious dosage, or when procedures may increase mechanical aerosolization and transmission of the agents by direct or indirect contact, then BSL3 should be considered (1,2,). Generally BSL3 is recommended for aerosol transmitted RG3 agents (1,2). However, when it is difficult to contract via aerosol due to the consistency of the biological specimen, analysis can be conducted in BSL2 with BSL3 practice and personal protective equipment (PPE) (4). In an outbreak situation, when the disease-causing agent is unknown, with severe or fatal infection, diagnosis of the agent should be at BSL3 or above with enhanced practice and equipment (5-9).

icddr,b constructed a BSL3 laboratory following a design provided by the National Institute of Health (NIH) to retrofit an existing laboratory in a 60 year old building. The facility and biosafety programme was successfully certified as compliant with the requirements of the 5th edition CDC/NIH BMBL (Biosafety in microbiological & biomedical laboratory) Guidelines and the CDC BSL3 Checklist (1,2). The BSL3 laboratory is divided into five sections (Figure 2a). The cascade of negative pressure maintained creates an inward directional airflow that does not allow release of any airborne particle from the BSL3 suite. The BSL3 suite has a redundant heating, ventilating and air-conditioning system to maintain constant temperature and pressure that is monitored by a central automatic control system in the control room. A supply side fan provides clean air (dust free) and the room air is filtered through high efficiency particulate filters, capable of trapping all infectious particles and preventing their release into the surrounding environment. The facility has a continuous surveillance system. The facility is equipped with all the recommended emergency systems (1,2). All the safety equipment, half of the room lights, and the emergency systems are under uninterrupted power supply (UPS). Additionally, the access control system has its own battery backup.

The BSL3 laboratory is suitable to culture, analyse antibiotic sensitivity, and isolate nucleic acid from BSL3 agents. Any article that enters the facility is removed after sterilization either by the autoclave or by surface sterilization with a suitable disinfectant. PPE, including gown, head cover, shoe cover, mask, gloves, and goggles, is mandatory in the BSL3 lab area to protect against the spread of infectious particles from personal belongings. The

changing room is in a semi-contaminated area and equipped to dispose of PPE. The autoclave room is in a clean zone where fresh PPE is stored and where any personal belongings are left here before entering into the hot (contaminated) zone. Sterilized waste material comes out from pass-through autoclave in the clean zone.

Figure 2a: Equipment and facilities at the icddr,b BSL3 laboratory



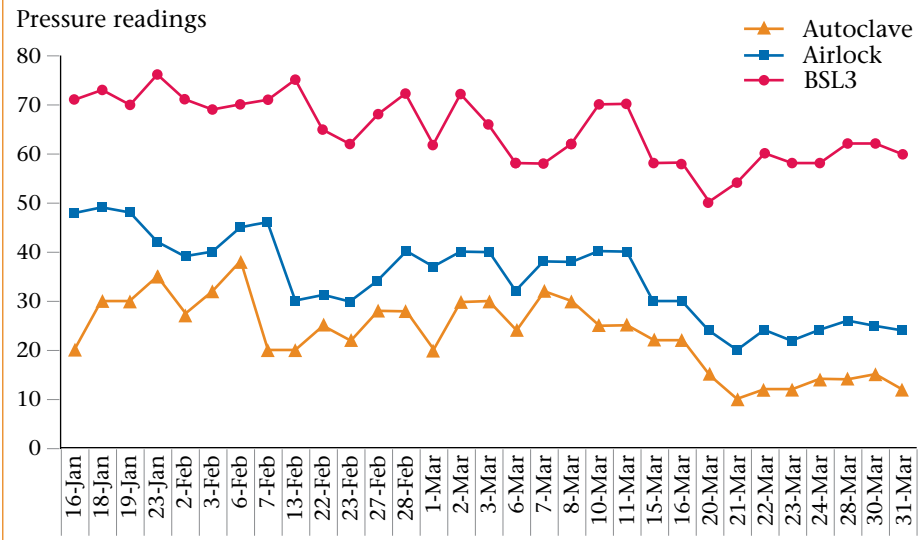
Equipment: Biosafety caninets [1], -80 freezer [2], refrigerator [3], MGIT [4], CO2 incubator [5], autoclave [6], pass-box [7], water bath [8], tabletop centrifuge [9], small incubator [10], micro-centrifuge [11], the sink with eyewash [12], camera [13].

BSL3 users participate in comprehensive biosafety training concerning the facility design, use of PPE, and standard operating protocol (SOP) for all activities. Each staff monitors and records the room pressure reading on the gauges at the entry door in a logbook while entering into the facility. Pressure readings from the month of January to March 2011 are shown for cascade of negative pressure that indicates the continuous maintenance of pressure differential among three sections in the laboratory (Figure 2b).

The laboratory is now being used approximately 2-6 hours every day, five days a week, by researchers analysing mycobacterium for bacterial identification, culture, nucleic acid isolation, and drug sensitivity analyses. Each day, 7-8 samples are analysed: among these, 10% have been found positive for pathogenic mycobacterium. Solid waste products are bagged in double biohazard bags and passed through the facility's pass-through autoclave

and then incinerated before disposal according to local guidelines. Liquid waste is decontaminated with 1% sodium hypochlorite. Reusable items are also passed through the pass-through autoclave and then laundered before reuse. All the work surfaces are routinely decontaminated before and after use with 0.5% hypochlorite. The biosafety cabinet surface is decontaminated with 1% hypochlorite followed with 70% ethanol to remove the residual hypochlorite that may corrode the stainless steel surface. The Biosafety Programme and the laboratory will be annually re-certified by a qualified third party.

Figure 2b: Maintenance of negative pressure cascade in three sections inside BSL3 laboratory



Reported by: Biosafety Programme and Biosafety Level 3 Laboratory, Laboratory Sciences Division, icddr,b.

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Comments

Diagnostics and research activities are being carried out in laboratories in Bangladesh with moderate and highly infectious agents with inadequate facilities. Currently there are two BSL3 facilities in Bangladesh (Figure 3), the certified facility at icddr,b and another facility, not yet in regular use, at the Institute of Epidemiology Disease Control and Research (IEDCR). These facilities can help in the early definitive diagnosis and further research on

important infectious diseases. We envision the BSL3 laboratory at icddr,b will contribute in three major areas: multi-drug-resistant tuberculosis, new strains of influenza, and diagnosis of specimens from outbreaks of unknown aetiology.

Figure 3: Distribution of containment laboratories worldwide

Two BSL3 laboratories, one at icddr,b and the other at IEDCR, are marked in Bangladesh. Only a few locations are disclosed due to security issues. A total of 1,356 CDC/USDA registered BSL3 facilities have been identified in 2007 throughout the United States (10). Elsewhere, Republic of Korea has 20, India has 16, Singapore has 10, Thailand has 5, Indonesia has 2, and Myanmar has 1.

Bangladesh is among the highest burden countries for tuberculosis (11). The emergence of dangerous new strains of *Mycobacterium tuberculosis*, including extremely drug-resistant strains represents a growing risk. As there is also an increased risk of co-infection between tuberculosis and HIV, an appropriately managed BSL3 laboratory can provide the ability to recognize, track and respond to emerging infectious diseases within Bangladesh.

Highly pathogenic avian influenza (HPAI) A virus subtype H5N1 has circulated continuously in poultry in Bangladesh since 2007. Three cases of human H5N1 have been confirmed in Bangladesh (8). Influenza H5N1-specific reverse-transcription polymerase chain reaction (RT-PCR) testing is conducted under BSL2 conditions. However, H5N1 viral culture, which is essential for more in-depth research, should be conducted under BSL3 enhanced conditions (5).

A large number of infectious disease outbreaks have been identified in various parts of Bangladesh during the last decade (6-8). Transporting specimens outside the country produces substantial time delays and represents a global biosecurity risk (9). Definitive safe diagnosis and characterization of outbreak specimens is

another national contribution that this BSL3 facility can make (9).

Capable microbiology laboratories are critical elements for disease surveillance, diagnosis, and prevention of infectious disease threats in this region that currently lack adequate facilities. The new certified BSL3 laboratory at icddr,b represents one step in improving Bangladesh's capacity to respond to infectious disease risks.

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Guillain-Barré syndrome in Bangladesh

To estimate the incidence of Guillain-Barré syndrome in Bangladesh in 2006 and 2007, in the <15 year old population, we used the Government's active surveillance for acute flaccid paralysis. We calculated that the crude incidence rate ranged from 1.5 to 1.7/100,000/year. During July 2006 to June 2007 we conducted a prospective case-controlled study in three tertiary medical college hospitals. Pure motor and axonal variants were the predominant forms of Guillain-Barré syndrome. *Campylobacter jejuni* was the predominant (57%) antecedent infectious agent in Guillain-Barré syndrome. The axonal variant of Guillain-Barré syndrome was associated with *C. jejuni* infection, severe residual disability, and high mortality.

Guillain-Barré syndrome is an acute polyradiculoneuropathy and the most frequent cause of acute flaccid paralysis in adults and children worldwide (1). The initial hallmark of Guillain-Barré syndrome is progressive muscle weakness with or without numbness. In some patients progressive shortness of breath indicates the involvement of respiratory muscles that can lead to respiratory failure. Double vision and difficulties with swallowing indicate cranial nerve involvement. Sensory symptoms include numbness, paresthesia, pain and ataxia. Pain is the initial symptom, and occurs more frequently in children. In some patients autonomic dysfunction causes blood pressure disturbances or cardiac arrhythmias. A symmetrical distribution of sensory and motor deficits is usually found during neurological examination, although asymmetric distribution of symptoms does not confirm the diagnosis. An increased protein level in cerebrospinal fluid is usually found by the second week of disease onset. Electrophysiology demonstrates three subtypes of Guillain-Barré syndrome: acute inflammatory demyelinating polyneuropathy; acute motor axonal neuropathy; and acute motor sensory axonal neuropathy. Approximately two-thirds of the patients report symptoms suggestive of a recent, preceding infectious illness before onset of symptoms of Guillain-Barré syndrome. *Campylobacter jejuni* is the most commonly identified antecedent infection

(2-4). *C. jejuni* infections are associated with a severe, pure motor, axonal variant of Guillain-Barré syndrome with a poor outcome (2,5,6). Infections with particular *C. jejuni* strains induce the production of cross-reactive antibodies to nerve gangliosides that cause nerve damage (7).

Bangladesh has achieved remarkable success to eradicate poliomyelitis. Since 2000 there has only been one cluster of 18 cases identified in 2006, which was linked to cases in India. However, the incidence rate of acute flaccid paralysis in Bangladesh in 2006-2007 was 3.25 per 100,000 children <15 year (8). This article summarizes a recently published article characterizing Guillain-Barré syndrome in Bangladesh (9,10). Our objective was to estimate the crude incidence rates of Guillain-Barré syndrome in Bangladesh in 2006 and 2007 obtained through acute flaccid paralysis surveillance among children <15 years of age, and to identify the preceding *C. jejuni* infection, clinical phenotype and prognosis of Guillain-Barré syndrome in a hospital-based prospective study.

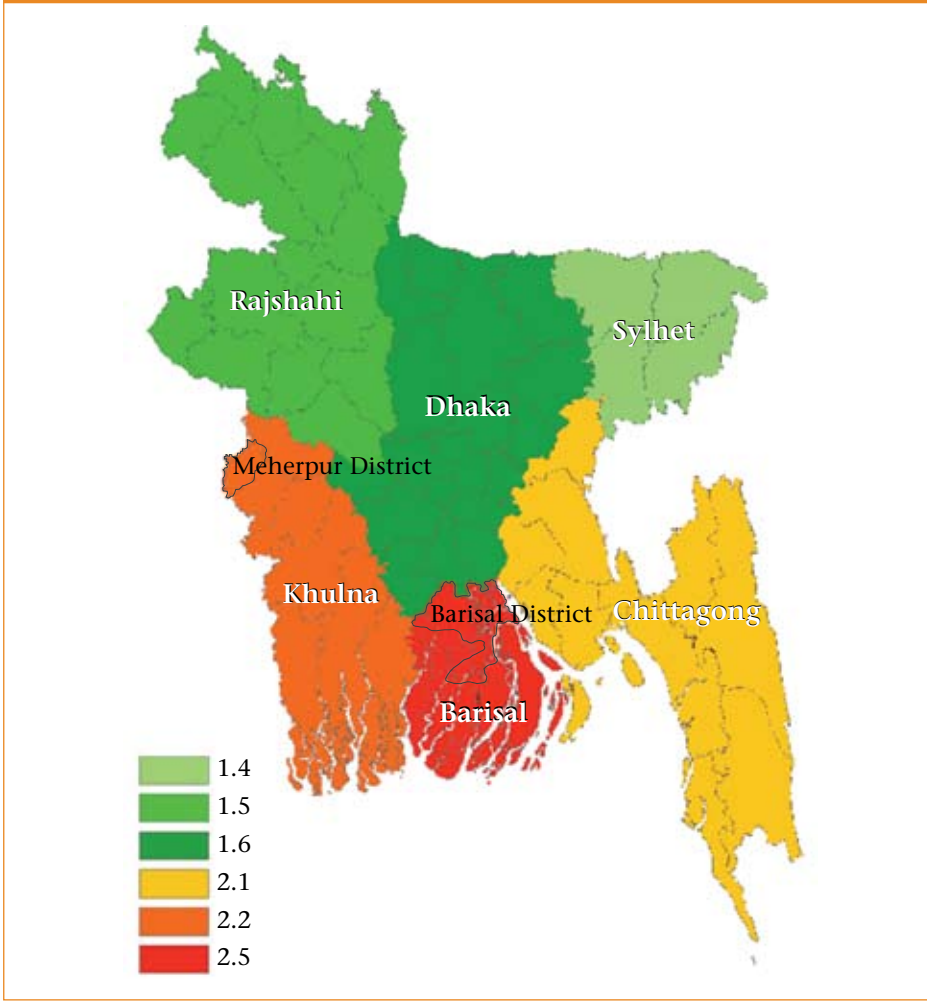
An ongoing active surveillance programme for acute flaccid paralysis is conducted by the Government of Bangladesh (GoB) in collaboration with the World Health Organization. Acute flaccid paralysis is defined as acute onset of focal or general flaccid (hypotonic) weakness without other obvious causes (e.g. trauma) in children <15 years. Based on clinical and other information routinely collected through the surveillance system, we defined a case of Guillain-Barré syndrome as a child <15 years of age with acute flaccid paralysis, absence of injury or birth trauma, and symmetrical paralysis. We calculated the crude incidence of Guillain-Barré syndrome in each of the six divisions and 64 districts of Bangladesh, based on cases of acute flaccid paralysis in the <15 year old population as reported by the GoB.

In 2006, the national surveillance system identified 1,619 cases <15 years with acute flaccid paralysis, of which 608 (37%) met the Guillain-Barré syndrome case definition. In 2007, 46% (855/1844) of cases of acute flaccid paralysis met the Guillain-Barré syndrome case definition. The crude incidence rate of Guillain-Barré syndrome in children <15 years ranged from 1.5 to 2.5/100,000/year in the six divisions. The crude incidence of Guillain-Barré syndrome in children <15 years ranged from 1.5 to 1.7/100,000/year in the three northern divisions, including Dhaka, Rajshahi, and Sylhet. In the three southern divisions, Khulna, Barisal and Chittagong, the crude incidence of Guillain-Barré syndrome in children <15 years ranged from 2.1 to 2.5 100,000/year (9). The highest incidence (>5.0/100,000/year) was observed in Meherpur and Barisal districts, in the southern part of Bangladesh (Figure 1).

We also conducted a prospective case-control study during July 2006 and June 2007, including 100 consecutive cases of Guillain-Barré syndrome admitted to three Dhaka hospitals: Dhaka Medical College Hospital;

Bangabandhu Sheikh Mujib Medical University; and Dhaka Central Hospital. Upon admission of a patient presumptively diagnosed with Guillain-Barré syndrome, a neurologist from Dhaka Medical College Hospital examined the patient within two days and confirmed the clinical diagnosis using the National Institute of Neurological Disorders and Stroke (NINDS) criteria for Guillain-Barré syndrome.

Figure 1: Crude incidence rate (per 100,000 per year) of Guillain-Barré syndrome in Bangladesh in 2006 and 2007 in children <15 years of age



Guillain-Barré syndrome affected predominantly young adult males living in rural areas (Table 1). More Guillain-Barré syndrome patients were identified

between January and March than at other times of the year (Figure 2). Sixty-nine percent of patients had clinical evidence of a preceding infection. The most frequent symptom was diarrhoea (36%). The majority of patients (92%) had a pure motor variant of Guillain-Barré syndrome with relatively infrequent cranial nerve involvement (30%). Twenty-five percent of patients required respiratory support. Electrophysiological studies demonstrated that 67% of patients had an axonal variant of Guillain-Barré syndrome. Eleven (14%) patients died and 23 (29%) remained severely disabled during the follow-up. Recent *C. jejuni* infection was identified by serology in 57% of Guillain-Barré syndrome patients as compared to 8% in family controls and 3% in control patients with other neurological diseases (P<0.001). *C. jejuni* infection was significantly associated with serum antibodies to the gangliosides GM1 and GD1a, axonal neuropathy, and greater disability. Poor outcome was significantly associated with the Guillain-Barré syndrome disability score at entry, presence of preceding diarrhoea, positive serology for recent *C. jejuni* infection and anti-GM1/GD1a antibodies.

Figure 2: Seasonal distribution of Guillain–Barré syndrome in Bangladesh, 2006 and 2007

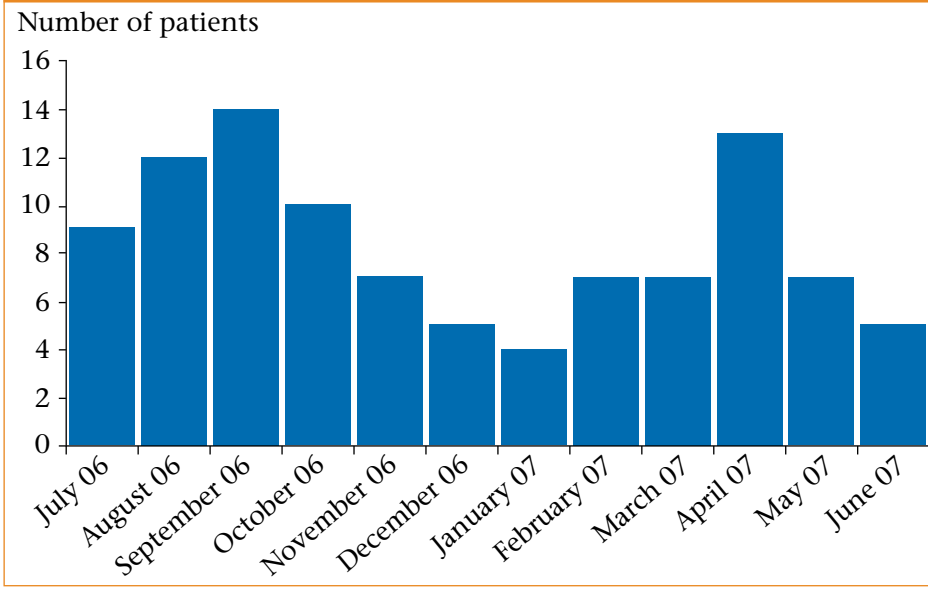


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Table 1: Demography, clinical and electrophysiological characteristics of Guillain-Barré syndrome patients

Demography	
Sex (M/F)	72/28
Median age, years (range)	21 (2.0-65.0)
Age categories (years)[1]	
<15	26 (26%)
16-30	47 (47%)
31-45	19 (19%)
>45	8 (8%)
Region	
Rural	71 (71%)
Urban	29 (29%)
Preceding symptoms and signs[2]	
Diarrhoea	36 (36%)
Respiratory symptoms	19 (19%)
Fever	14 (14%)
Cerebrospinal fluid (N=78)	
Protein level >40 mg/dl	77 (99%)
Cell count <15 cell/µl	78 (100%)
Neurological symptoms (cranial nerve impairment)	
Facial nerve palsy	25 (25%)
Oculomotor palsy	4 (4%)
Lower bulbar palsy	10 (10%)
None	70 (70%)
Sensory deficits	8 (8%)
Pain	10 (10%)
MRC sum score (at entry)[3]	
60-51	8 (8%)
50-41	21 (21%)
40-31	12 (12%)
30-21	19 (19%)
20-0	40 (40%)
Guillain-Barré syndrome disability score (at entry)	
1 or 2	11 (11%)
3	11 (11%)
4	53 (53%)
5	25 (25%)
Classifications of Electrophysiology (N=64)	
AMAN[4]	36 (56%)
AMSAN[5]	7 (11%)
AIDP[6]	14 (22%)
Unclassified[7]	7 (11%)

[1]Age divided into 4 categories: 1st age group was <15 years based on AFP surveillance; others were grouped by 15 years intervals.
[2]Symptoms of an infection in the last 4 weeks preceding the onset of weakness.
[3]Medical Research Council sum score was defined as the sum of six muscles in the upper and lower limbs on both sides, ranging from 60 (normal) to 0 (quadriplegic).
[4]AMAN: acute motor axonal neuropathy; [5]AMSAN: acute motor sensory axonal neuropathy; [6]AIDP: acute inflammatory demyelinating polyneuropathy; [7]Unclassified: not fulfilling criteria for AMAN, AMSAN, AIDP

Comment

While ongoing surveillance for acute flaccid paralysis demonstrates the absence of polio circulating in Bangladesh, this study confirms that a large proportion of children with acute flaccid paralysis have Guillain-Barré syndrome. A recent review reported that the overall incidence of Guillain-Barré syndrome varies between 1.1/100,000/year and 1.8/100,000/year in different geographical areas of the world (11). The crude incidence rate of Guillain-Barré syndrome in Bangladesh in the <15 year old population appeared 2.5 to 4 times higher compared to available data from other countries (9). In addition, our prospective case-control study suggests that the majority of the patients were males, less than 30 years old, and suffering from a severe, pure motor and axonal variant of Guillain-Barré syndrome. We found a strong association with preceding *C. jejuni* infection, in particular in those cases diagnosed with acute motor axonal neuropathy. The frequency of *C. jejuni*-infected Guillain-Barré syndrome is higher than in high income countries. It is possible that people in rural Bangladesh have a particularly high level of exposure to *C. jejuni*, which may contribute to the high incidence of Guillain-Barré syndrome. *Campylobacter* spp. are widespread in the environment and constitute part of the natural intestinal flora of many mammalian species and birds (12). In high income countries, poultry meat contaminated with *C. jejuni* is believed to be the primary vehicle of human infection; transmission is thought to occur either as a result of cross-contamination due to improper handling of raw meat or consumption of undercooked food (12). In Bangladesh poultry exposure is widespread. Sixty one percent of rural households in Bangladesh raise poultry (13), and so rural residents may have high exposure to *C. jejuni*.

Patients with Guillain-Barré syndrome in Bangladesh have a much poorer prognosis: 43% die or have residual severe disability compared with 20% of patients in high income countries (10,14). The poor prognosis may be explained in part by the high frequency of *Campylobacter* diarrhoea and the disease severity at onset, two known independent predictors for a poor prognosis (14), as well as malnutrition, which is common in Bangladesh. In addition, the majority of patients do not receive specific IVIg treatment, as the cost of 1 million Taka per adult patient, is prohibitively expensive.

Although *Campylobacter* infections in Bangladesh appears to play a prominent role in the pathogenesis Guillain-Barré syndrome, other agents may also be involved. Cutting-edge microbial discovery laboratory platforms and modern and powerful high-throughput sequencing tools may detect other, yet unknown, pathogens that intervene in the pathogenesis of Guillain-Barré syndrome. Antibodies against other glycoconjugates may be present in the *Campylobacter* negative Guillain-Barré syndrome patients. Also, new and affordable treatment regimens should be explored as the current standard treatment is expensive and therefore not an option for affected people in

Bangladesh and other low-income countries. An improved understanding of how people become infected with *C. jejuni* infection in Bangladesh may also identify opportunities to interrupt transmission.

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The 2011 cholera outbreak in Kishorganj

In April 2011, a joint investigation team from IEDCR and icddr,b investigated an outbreak of acute watery diarrhoea in Kishorganj district. Eighty-four patients from three urban communities of Sadar Upazila developed diarrhoea between 10 and 19 April, 2011 and were admitted in the Kishorganj District Hospital. The onset of severe watery diarrhoea in adults and isolation of cholera organisms from their rectal swabs confirmed that the outbreak was caused by *Vibrio cholerae*. The detection of *Vibrio cholerae* in collected tap water samples from case households suggested water contamination in the pipelines as the most likely souce of this outbreak. Most cases reported that they drank tube well water, but they used tap water for washing food and utensils, cooking, and poured it into tube wells to raise the water pressure when the water levels were low. Research exploring effectiveness of water purification strategies including chlorination in the context of smaller municipalities with intermittent water supply could help identify appropriate approaches for ensuring safe water supply at the municipal level.

Vibrio cholerae is responsible for an estimated three to five million cases worldwide with an estimated 120,000 deaths per annum and remains a major public health challenge to many low income countries with poor access to safe water and proper sanitation, including Bangladesh (1,2). Although cholera cases in Bangladesh were not reported to the World Health Organization until recently, reports of cholera from Bangladesh are commonly published in the scientific literature (3-8). Expert estimates suggest an incidence of about 450,000-1000,000 cases of cholera in Bangladesh each year (9).

On 17 April 2011, newspapers reported a diarrhoea outbreak in three municipal areas of an urban sub-district of Kishorganj. Later that day the Civil Surgeon of Kishorganj district also reported a sudden rise in the number of patients admitted with severe acute watery diarrhoea to the District Hospital from 16 April 2011 and requested assistance from the Institute of Epidemiology, Disease Control and Research (IEDCR) of the Government of Bangladesh to determine the cause of the outbreak. An outbreak investigation team from IEDCR and the International Centre for Diarrhoeal Diseases Research, Bangladesh (icddr,b) went to the affected district on 19 April, 2011 to identify the cause of the outbreak, the pathway of transmission and to propose control measures.

The outbreak investigation team visited the Civil Surgeon's office and collected preliminary information from the District Sanitary Inspector regarding the residents, their socioeconomic status, their drinking water

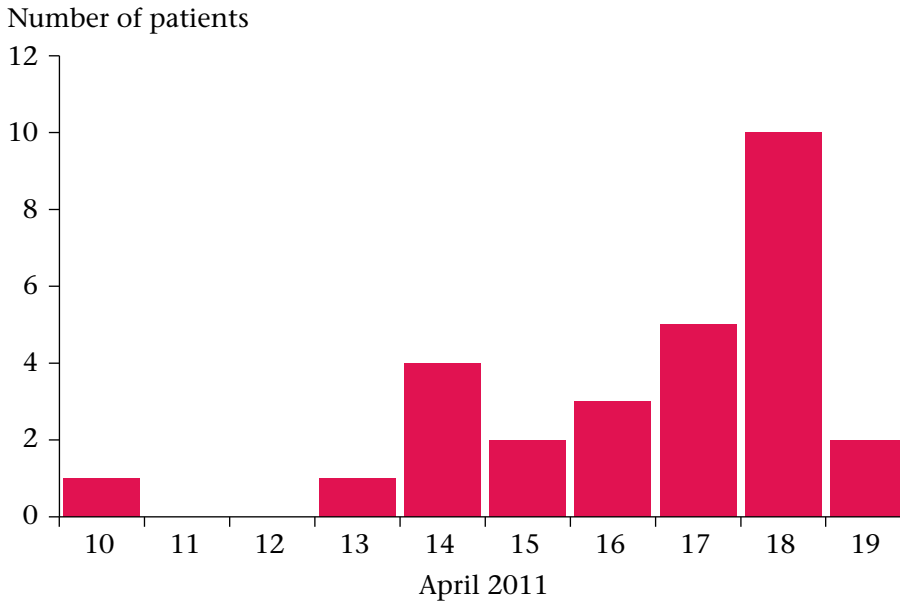
supply and the sanitation situation. The team then visited the Kishorganj District Hospital and collected information from some selected cases and care-givers, including hospitalized cases with the earliest dates of onset of illness, local health officials, including the Resident Medical Officer (RMO), hospital physicians, nurses and paramedics providing care to admitted patients to understand the nature of the illness outbreak. The team also visited the affected areas of Kishorganj to explore the sanitation and water distribution systems.

We defined a case of outbreak associated severe diarrhoea as any resident of Kishorganj district admitted with acute watery diarrhoea (passage of ≥3 loose stools per 24 hours) to the Kishorganj District Hospital since 16 April 2011. We identified 84 cases of outbreak-associated severe diarrhoea admitted between 16 and 19 April, 2011 (Figure 1). Among them 52% (44/84) were males with a median age of 21 (range: 4 months-80 years). We interviewed 22 cases who were admitted in the hospital during the period of investigation using a pre-tested, standardized case investigation form to collect exposure and clinical histories. We visited two (Tarapsha and Boila) of the three affected communities (Tarapasha, Boila and Shatal) of ward seven of Kishorganj municipal area. To identify the possible source of the outbreak, we interviewed four cases, including the index case from Tarapasha, about their exposures. We also interviewed two cases from households having at least three affected members from Boila, all of whom were discharged from the hospital. The health workers from the municipality and the health sector looked for additional severe cases or deaths associated with watery diarrhoea and reported them to the Civil Surgeon. We requested the Civil Surgeon to report any additional admissions and/or deaths to IEDCR on a daily basis for one week following the investigation to IEDCR. There were no reported additional cases after 19 April and no reported deaths.

Of the 28 cases of acute watery diarrhoea interviewed, 46% (13/28) were males; their mean age was 22 years (median: 10 years; Range: 4 months-75 years). Among them, 39% lived in Tarapasha, 32% lived in Shatal and 18% lived in Boila. Seventy-nine percent (22/28) of the patients were admitted in the District Hospital within 24 hours of onset of symptoms. All of the interviewed cases reported sudden onset of acute watery diarrhoea that later became mixed with mucous. Half of the cases reported passing stools >50 times on the day of admission; 39% (11/28) reported abdominal cramping; and 75% (21/28) also reported vomiting more than once (range: 1-15). Based on the diarrhoea treatment guideline of the World Health Organization (WHO), the attending physician classified five patients as having 'no dehydration', 15 with 'some dehydration', and eight with 'severe dehydration' at the time of admission. Physicians reported that the physical condition of the case patients improved with appropriate resuscitation, including intravenous cholera saline and antibiotics, after admission. Sixty-eight percent (19/28)

of cases interviewed had at least one other family member who experienced similar symptoms since 10 April 2011, and 43% (12/28) of cases had two or more members affected with similar symptoms (range: 0-8). Thirty-two percent (9/28) mentioned that they used municipality supplied tap water for drinking, while 68% (19/28) reported that they drank only tube well water. However, people always poured 2-3 litres of tap water into the tube wells to initiate water flow as the tube wells did not work during the summer when the water level was low (Picture 1: see page 25). Moreover, about half (54%) of all cases mentioned that they used tap water for washing raw foods, cooking, washing utensils and bathing. Only one case (4%) reported boiling her drinking water.

Figure 1: Onset of illness of acute watery diarrhoea cases admitted in the District Hospital, Kishorganj, 2011 (N=28)



We collected rectal swabs from 18 outbreak associated severe diarrhoea cases admitted in the District Hospital and two cases from the community and transported them in bacterial transport media to IEDCR's Microbiology Laboratory where they were tested for *Vibrio cholerae* O1 and O139, *Shigella* spp., *Salmonella* spp. and parasites. Only *Vibrio cholerae* Ogawa was isolated from eight (40%) of the 20 samples.

Common community perceptions regarding the cause of illness included the recent heat wave and the increased number of flies in households following insecticide spraying of the open sewers by the municipality. Other residents

perceived that the dirty and smelly water coming out of taps from a recently repaired municipal pump could have caused the illness. The municipality's pump, which supplied the affected areas, had not been functional for the last three months, but had been repaired about one week before the outbreak. Though people knew that water could be contaminated and that water purification strategies, including boiling, could prevent water-borne diseases, none actually boiled or chemically purified drinking water. Some felt that water became *dead* (stale) and tasteless after boiling.

Almost all patients initially tried home-based management of the illness with oral rehydration salts. However, they went to qualified private or public practitioners when their condition deteriorated. Some physicians expressed the need for regular training on the recent management guidelines for acute watery diarrhoea, including cholera. This is because antibiotic resistance is common, the guidelines about which specific anitibiotics should be used change frequently, and physician turnover is rapid. Physicians reported a shortage of necessary antibiotics and rice-based oral saline, although oral rehydration salts and intravenous cholera salines were available in the hospital.

We conducted key informant interviews with a group of health workers, local leaders, and the Chief Engineer of the Department of Public Health Engineering (DPHE) of Kishorganj to collect information regarding local water supply and sanitation. Ground water is drawn through a deep tube well of the DPHE and then directly distributed to households through an interconnected piped water system, without any treatment. The deep tube well and pipelines are maintained by the municipal authority. Water is supplied intermittently for about one and a half hours three times daily. The authorities also reported that they had recently repaired the pump supplying the affected areas, which was not functioning for about three months. They neither cleaned the pipes nor treated the supplied water following repair. We visited the pump site and found no visible leakages. However, pipelines and tube wells were in close proximity to sewerage lines and toilets and water distribution pipelines passed through open sewers in some areas (Picture 1: see page 25).

We collected water samples from selected case household taps, shallow tube wells present in some of the affected households, one affected household's deep tube well, the municipality's pump and the central pump of the Kishorganj DPHE. All collected samples were tested within 24 hours of collection in icddr,b's Environmental Microbiology Laboratory. We isolated *Vibrio cholerae* O1 (Ogawa) from both of the municipal supplied tap water samples. Total coliforms were isolated from six of the nine water samples and faecal coliforms were isolated from five out of the nine water samples (Table 1).

Reported by: Institute of Epidemiology, Disease Control and Research (IEDCR) and Centre for Communicable Diseases, icddr,b.

Table1: Bacteriological test results of water samples collected from various sources

Points of water collection	Number of samples exceeding the limits of organisms, (Range of organisms)					
	Total coliforms	Faecal coliforms	*Vibrio cholerae*	*Aeromonas* spp.	*Pseudomonas* spp.	*Salmonella/ Shigella* spp.
Tube well (N=5)	2, (0-2000)	2 (0-1000)	0, (0)	2, (1)	1, (1)	0, (0)
Municipal supplied tap water (N=2)	2, (130-176000)	2, (147-77000)	2, (*V. Cholerae* o1 Ogawa)	2, (1)	1, (1)	0, (0)
Department of Public Health & Engineering pump (N=1)	1, (8)	0, (0)	0, (0)	1, (1)	0, (0)	0, (0)
Municipal pump (N=1)	1, (252)	1, (105)	0, (0)	1, (1)	1, (1)	0, (0)

Maximum allowable limit by World Health Organization's Guideline is 0 CFU/100 ml for the organisms: Total coliforms, faecal coliforms, *Vibrio cholerae, Aeromonas* spp., *Salmonella* spp., *Shigella* spp., *Pseudomonas* spp.

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Comments

The clinical features of onset of severe watery diarrhoea in adults and isolation of pathogenic strains of cholera organisms in 40% of the rectal swab samples collected from suspected cases confirm that this outbreak of acute watery diarrhoea affecting three municipal areas of a sub-district of Kishorganj was caused by *Vibrio cholerae*.

The public health burden of cholera is significant and it is a notifiable disease under the International Health Regulations. Yet, only five cholera outbreaks have been reported during the last five years (10) through the governmental system. In contrast, the quarterly journal of icddr,b's Health and Science Bulletin regularly reports thousands of cholera cases from the two icddr,b hospitals in Dhaka and Matlab.

Unlike the majority of the previous cholera outbreaks (10,11), we found no deaths during this outbreak. The affected area's close proximity to the District Hospital and all affected cases' ability to seek care from qualified practitioners suggest that prompt appropriate management assisted recovery of cholera affected patients.

The sudden clustering of diarrhoea cases within three municipal areas of Kishorganj following a heat wave (7) with a suspected common exposure, and the detection of high levels of total coliforms and *Vibrio cholerae* in the

collected tap water samples, suggest water contamination in the pipelines as the most likely source of this outbreak. Similar sources have been reported in cholera outbreaks during the past two years from urban communities in Pabna, Tangail and Bogra with piped water systems lacking chlorination (10-12). Although 68% of the affected cases mentioned drinking only tube well water, more than half (54%) of all affected cases used municipal supplied tap water for household and personal purposes including washing foods, washing utensils, cooking and bathing. In addition, household tube well water may also get contaminated with cholera organisms from tap water poured into the wells to raise the water levels. Once tap water is contaminated with cholera organisms, the opportunities for spread by contaminated fruits, vegetables, raw or semicooked foods are also greatly increased (13).

Although diarrhoeal diseases cause significant mortality and morbidity in the country (14) and the World Health Organization recommends chlorination of the piped water and boiling or chemical treatment of water at home for prevention of outbreaks of enteric infections in urban communities (15-17), the piped water was neither chemically treated nor was drinking water boiled and/or chemically purified at home. The prevailing low risk perception in Bangladeshi communities regarding water safety might translate into less pressure on governmental authorities to address water purification issues, which, in turn puts communities at ongoing risk for outbreaks of water borne illnesses.

One limitation of this investigation was the focus on hospitalized cases, so that the number of cases reported as part of this outbreak is likely an underestimate. As we did not conduct a robust house-to-house search in the community, we might have failed to detect deaths from cholera within the community. Moreover, approximately half of the cases surveyed had two or more family members who developed similar symptoms, which suggests that the total community burden of cholera was likely greater than only the hospitalized cases.

The successful containment and control of cholera outbreaks requires a coordinated and combined approach including: prompt outbreak investigation; adequate laboratory facilities for isolation and detection of microbial resistance; the availability of rice-based saline and antibiotics; prompt preventive measures; and trained health care professionals at all levels of healthcare. The DPHE and municipal authorities need to maintain pipelines and ensure a continuous flow of piped water so that negative pressure will not draw in contaminants through any leaks during intermittent supply. As an immediate preventive strategy, affected communities should treat at least their drinking water at the point of use by boiling, adding chlorine or other appropriate measures, which would reduce the exposure significantly. However, community members reported disliking the taste of boiled water which could make them less likely to adopt this strategy.

Research exploring new approaches to providing safe water in smaller municipalities with intermittent water supply and purification strategies targeted after repair operations may ultimately provide feasible, low-cost solutions for microbiologically safe water. Improved surveillance for cholera by the government health sector could detect outbreaks more frequently, which, in turn could help focus more attention on steps that need to be taken to improve the water supply infrastructure to reduce the burden of water-borne diseases.

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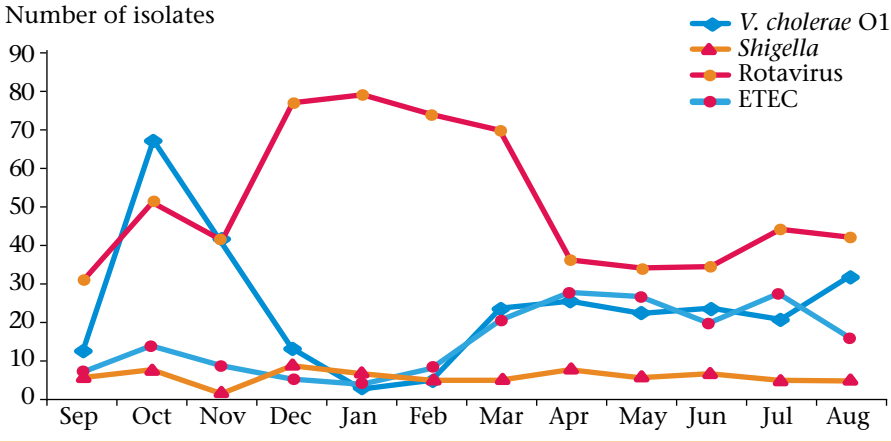
Surveillance updates

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.

Proportion of diarrhoeal pathogens susceptible to antimicrobial drugs: September 2010-August 2011

Antimicrobial agents	*Shigella* (n=73)	*V. cholerae* O1 (n=294)
Nalidixic acid	Not tested	Not tested
Mecillinam	65.8	Not tested
Ampicillin	60.3	Not tested
TMP-SMX	30.6	1.0
Ciprofloxacin	49.3	98.3
Tetracycline	Not tested	57.8
Erythromycin	Not tested	100.0
Furazolidine	87.7	Not tested

Monthly isolation of* V. cholerae *O1*, Shigella, *Rotavirus and ETEC September 2010-August 2011



Antimicrobial resistance patterns of 145 M. tuberculosis *isolates: August 2010-July 2011*

Drugs	Resistance type		Total n=145 (%)
	Primary n=133 (%)	Acquired* n=12 (%)	
Streptomycin	21 (15.8)	3 (25.0)	24 (16.6)
Isoniazid (INH)	4 (3.0)	1 (8.3)	5 (3.4)
Ethambutal	1 (0.8)	1 (8.3)	2 (1.4)
Rifampicin	4 (3.0)	1 (8.3)	5 (3.4)
MDR (INH+Rifampicin)	1 (0.8)	1 (8.3)	2 (1.4)
Any drugs	24 (18.0)	3 (25.0)	27 (18.6)

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

Antimicrobial susceptibility pattern of S. pneumoniae *among children <5 years during July-September 2011*

Antimicrobial agents	Total tested (n)	Susceptible n (%)	Reduced susceptibility n (%)	Resistant n (%)
Ampicilin	2	2 (100.0)	0 (0.0)	0 (0.0)
Cotrimoxazole	2	1 (50.0)	0 (0.0)	1 (50.0)
Chloramphenicol	2	2 (100.0)	0 (0.0)	0 (0.0)
Ceftriaxone	2	2 (100.0)	0 (0.0)	0 (0.0)
Ciprofloxacin	2	2 (100.0)	0 (0.0)	0 (0.0)
Gentamicin	2	0 (0.0)	0 (0.0)	2 (100.0)
Oxacillin	2	2 (100.0)	0 (0.0)	0 (0.0)

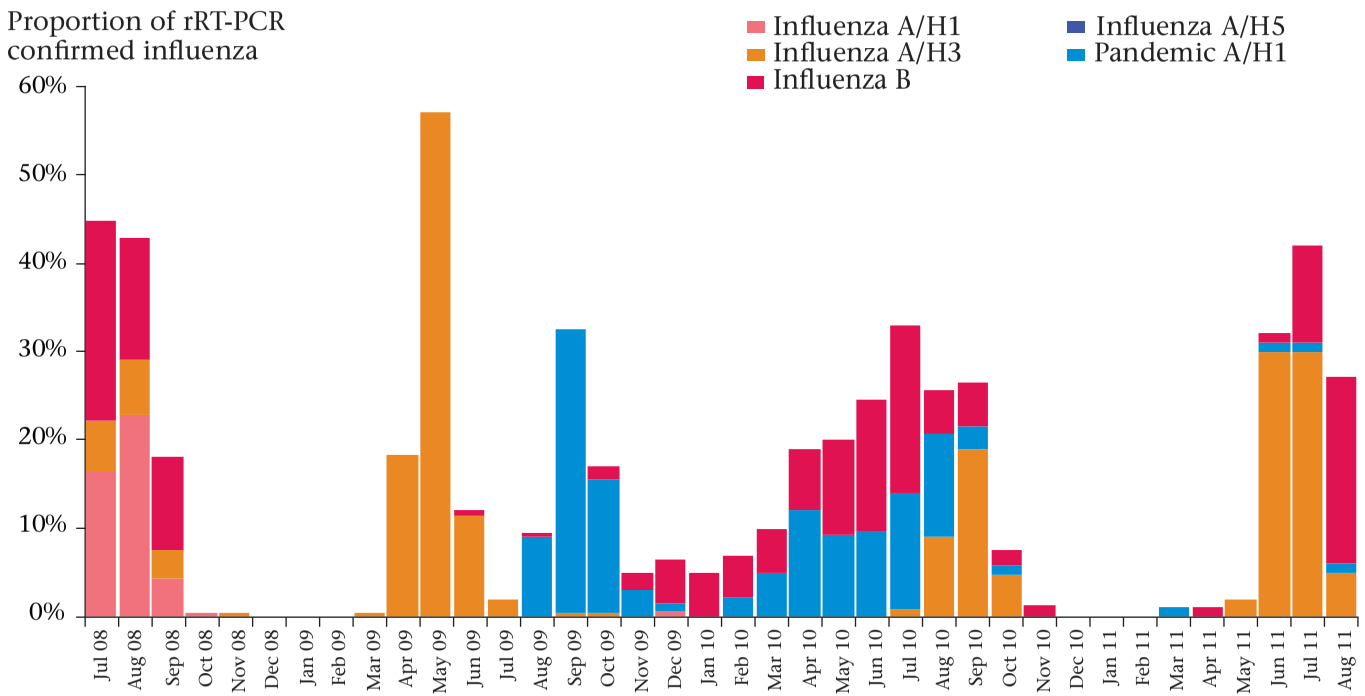
Source: icddr,b's urban surveillance in Kamalapur (Dhaka).

Antimicrobial susceptibility pattern of S. typhi *among children <5 years during July-September 2011*

Antimicrobial agents	Total tested (n)	Susceptible n (%)	Reduced susceptibility n (%)	Resistant n (%)
Ampicilin	23	13 (56.5)	0 (0.0)	10 (43.5)
Cotrimoxazole	23	14 (60.9)	0 (0.0)	9 (39.1)
Chloramphenicol	23	14 (60.9)	0 (0.0)	9 (39.1)
Ceftriaxone	23	23 (100.0)	0 (0.0)	0 (0.0)
Ciprofloxacin	23	0 (0.0)	23 (100.0)	0 (0.0)
Nalidixic Acid	23	1 (4.3)	0 (0.0)	22 (95.7)

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 July 2008 and August 2011



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)

Picture 1: A women washing fish with municipal tap water flowing through a pipeline submerged in an open sewer (left) and a woman pouring tap water into the tube-well to raise water level for drawing tube-well water (right)

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