

Shigellosis: Report of a Workshop*

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INTRODUCTION

Shigellosis continues to be a major public-health problem and remains endemic in many developing countries. Among *Shigella* species, *Shigella dysenteriae* type 1 poses a particular threat because of the severity of disease and its epidemic potential. Epidemics due to *S. dysenteriae* type 1 tend to occur at intervals of about a decade, and although few outbreaks have been reported over recent years, the problem should not be considered to be solved. There is no reason to believe that new outbreaks will not occur in the future because the same conditions that prevailed during outbreaks in the past are still present and include over-crowding, poor sanitation, sub-standard hygiene, and unsafe water supplies, while no other prevention measures have been developed in the interim.

Efforts to control shigellosis should include measures targeting behaviour, personal hygiene, sanitation, and water supply, in addition to proper case management

of patients. Until now, fluoroquinolones, such as ciprofloxacin and norfloxacin, have been active against *Shigella*, but outbreaks due to *S. dysenteriae* type 1 strains, resistant to these antibiotics, have been documented recently. If past patterns hold true, new epidemics of shigellosis due to fluoroquinolone-resistant *S. dysenteriae* type 1 may be expected during the coming years, particularly in South Asia. Detection of past epidemics has often been delayed, and the antibiotic sensitivity profile of the strains was often either ignored or not appreciated. Improved epidemiological surveillance systems are needed to detect these outbreaks, determine the antibiotic sensitivity patterns, and prepare for interventions.

In this context, a workshop on shigellosis was jointly organized by the World Health Organization (WHO), ICDDR,B: Centre for Health and Population Research, the International Vaccine Institute (IVI), and United States Agency for International Development. Representatives from research laboratories and public-health

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professionals from both developing and industrialized countries were present. The purpose of the workshop was to:

- a. review the burden of dysentery from different regions of the world;
- b. review surveillance techniques for shigellosis;
- c. prepare recommendations for controlling endemic and epidemic shigellosis (WHO guidelines); and
- d. define research priorities which will lead to improved methods for controlling shigellosis

The workshop emphasized improved surveillance and practical interventions for control.

BURDEN OF DISEASE

The burden of disease in Asia was estimated by reviewing studies initiated in Asian countries after 1990, and of which the results were published. Total morbidity and mortality attributable to *Shigella* were estimated through extrapolation. Overall, shigellosis remains a common disease in the continent. The annual numbers of episodes and deaths due to *Shigella* in Asia were estimated to be 91 million and 414,000 respectively. *S. flexneri* is the commonest serotype, followed by *S. sonnei*. Resistance to common antibiotics is frequent and is alarming in the case of *S. dysenteriae* type 1.

The Diseases of the Most Impoverished (DOMI) Programme of IVI is currently conducting shigellosis disease-burden studies in Bangladesh, China, India, Indonesia, Pakistan, Thailand, and Viet Nam. Methods include retrospective collection of existing data, prospective surveillance, and behavioural and economic studies. Overall, a population of 568,000 has been placed under surveillance for two years. Data have been collected so far from four sites. Morbidity due to shigellosis was higher than expected: 64,755 episodes of diarrhoea have so far been detected, including 3,304 episodes of shigellosis. The overall crude incidence rates of shigellosis ranged from 1 to 12/1,000 per year, and the case-fatality rate was as low as 1.5 per 1,000 *Shigella*-associated episodes. However, these figures were corrected to take into account the lack of sensitivity of the culture techniques and patients escaping the passive surveillance network. The predominant *Shigella* species was *S. flexneri*, except in Thailand (where it was *S. sonnei*). An unexpected serotype heterogeneity and untypability was found in *S. flexneri*, which is a

cause of considerable concern for vaccine developers. *S. dysenteriae* type 1 has not been isolated so far at any surveillance site. More than 50% of isolates were resistant to ampicillin, and a similar proportion was resistant to co-trimoxazole. High resistance to nalidixic acid was observed in Bangladesh and China.

Another review aimed at estimating the burden of shigellosis and the evolution of antimicrobial drug resistance of *S. dysenteriae* type 1 over the past 10 years in sub-Saharan Africa. The analysis did not reveal any trends over time towards a reduction in burden of shigellosis on the continent. A first wave of outbreaks erupted in the Great Lakes region in 1993-1994. The first reported epidemic due to *S. dysenteriae* type 1 in West Africa occurred in Abidjan in 1998. The West African region was further affected by numerous outbreaks in 1999 and 2000. Surveillance data collected from 11 countries revealed a median incidence rate of bloody diarrhoea of 10.2/1,000 per year. The number of bloody diarrhoea cases seen at health facilities in Africa can be estimated to reach more than 8 million per year. Resistance of *S. dysenteriae* type 1 to nalidixic acid is more common in countries of the Great Lakes region than in Southern and Western Africa. However, the clinical efficacy of nalidixic acid has been questioned for many years, even against sensitive strains of *S. dysenteriae* type 1. Experiences from Asia and from the Great Lakes region demonstrate that *S. dysenteriae* type 1 develops resistance within a few years of intense use of this drug, as was the case with previous antimicrobials used in shigellosis. Poor effectiveness of nalidixic acid should, therefore, be expected in future outbreaks on the African continent. More sensitive diagnostic tools and better epidemiological surveillance systems would reduce the delay in detection of outbreaks. Laboratory capacities should be reinforced to better monitor the antimicrobial sensitivity pattern of *S. dysenteriae* type 1 during and between the periods of epidemics.

UPDATE ON EPIDEMIOLOGY AND TRANSMISSION OF SHIGELLOSIS

Since 1980, ICDDR,B has maintained a systematic surveillance system for diarrhoeal diseases among hospitalized patients in Dhaka. *Shigellae* have been isolated from about 10% of patients during this time, with *S. flexneri* being the most common species. Overall, shigellosis tends to occur more frequently before and after the rainy season, and the *Shigella*-peak season seems to follow the cholera season by a few weeks.

Rates of infection due to *S. dysenteriae* type 1 appear to follow a 10-year cycle, and with each new cycle, the epidemic strain seems to acquire resistance to the antibiotics that were commonly used for treatment. Thus, nearly all strains of *S. dysenteriae* type 1 are now resistant to nalidixic acid and to commonly-used antibiotics. Recently, outbreaks of dysentery due to *S. dysenteriae* type 1, resistant to ciprofloxacin and the other newer quinolones, have been observed in India, Bangladesh, and Nepal. It is possible that these outbreaks herald the onset of a new epidemic of shigellosis due to strains resistant to ciprofloxacin. New findings from environmental studies demonstrate that *Shigellae* can be found in environmental water samples, including at times when no cases of *S. dysenteriae* were being isolated from patients.

In refugees and internally-displaced population (IDP), the risk of outbreaks of shigellosis is high because of an insufficient supply of clean water, poor sanitation, and over-crowding. *S. dysenteriae* type 1 has been responsible for large outbreaks, particularly in Central and Southern Africa, in the 1990s. In such settings, *Shigella*-associated outbreaks are reported with higher attack rate and case-fatality rate. For example, in Goma (DRC), in July 1994, the attack rate of the *S. dysenteriae* type 1-associated epidemic was estimated at between 8% and 12%, with a case-fatality rate of 30%. Particular attention should be given to offering an appropriate response to outbreaks due to *S. dysenteriae* type 1 among refugees and internally-displaced populations, providing access to diagnostic capacities and effective antibiotics (short treatment regimen, affordable, adapted to the resistance pattern). The optimal strategy would be to provide antibiotic treatment to all dysentery cases and to hospitalize severe cases. A supplementary feeding programme should also be implemented.

Shigella is transmitted typically from person to person. Several investigations demonstrated that the transmission of the disease increases with poor hand-hygiene, contaminated drinking-water, inadequate sanitation, and poor toileting behaviours. Indeed, poor toileting practices may lead to contamination of hands and subsequently of stored drinking-water and food. However, epidemiologic investigations suggest that intra-familial transmission accounts for only a minority of dysentery cases. Interventions to prevent the transmission of *Shigella* include improving hand-hygiene, ensuring quality of drinking-water with point-of-use water disinfection and safe water storage, improving sanitation conditions, altering toileting practices to minimize contact

between hands and stool, and fly control where appropriate. Numerous studies have documented that the use of soap and water to wash hands results in a significant reduction in diarrhoea risk. Chemo-prophylaxis of contacts of cases is strongly discouraged.

Although the need for a *Shigella* vaccine is urgent, progress to date has been hampered by the antigenic complexity of *Shigella* species, the lack of cross-protective epitopes among the different species, and the lack of the understanding of the protective immune response. Several approaches have been used, the most advanced being the use of live-attenuated *Shigella* strains. Several candidates are based on *S. flexneri* 2a, and one is based on *S. sonnei*. Volunteer studies have shown that the vaccine candidates SC602 and CVD1203 against *S. flexneri* 2a, and WRSS1 against *S. sonnei* were immunogenic and protective when challenged with the homologous strain. Unfortunately, higher immunogenic doses were associated with severe adverse events, and further evaluation of these vaccine candidates is warranted. Another approach has been to include live carrier strains, such as *Escherichia coli*, to deliver the lipopolysaccharide O antigen of *Shigella*. An initial evaluation of clinical trials has shown that these strains are immunogenic. A variation of this approach is to deliver a mixture of *Shigella* strains. Conjugate vaccines have been developed and evaluated in phase I and II volunteer studies. Three vaccine candidates have demonstrated immunogenicity and efficacy in adults. Thus, although there is much progress, the challenges remain the same. The challenges are: which strains to include in a vaccine candidate for global use?; what are the attenuating mutations needed in the live strains?; what are the protective immunological mechanisms and the correlates of protection? Furthermore, the vaccine candidates need to be evaluated in clinical trials in children in endemic regions.

The DOMI Programme of IVI conducted socio-behavioural research in the domain of shigellosis, typhoid fever, and cholera. Both qualitative and quantitative methods are used. The objective is to understand the perceptions, attitudes, knowledge, and experience of the local populations regarding these diseases and vaccination. Data can be used for improving the design and implementation of vaccine-effectiveness trials, increase participation in these trials, and provide information for policy-makers in relation to future campaigns. Data show important issues to be considered for future introduction of a public-health campaign

against shigellosis, especially for a vaccine. Individuals in the 'low-demand' category are most likely to waver between the decision to receive or not to receive the vaccine. While the majority of policy-related decisions must take place on a per-country basis, there are some potential common attributes of both 'refusers' and 'low-demand' individuals across the countries surveyed which are important to consider before the introduction of a public-health campaign against shigellosis on a regional basis.

SURVEILLANCE TECHNIQUES FOR SHIGELLOSIS

Historically, non-motile, anaerogenic, auxotrophic bacteria, resembling *E. coli* and associated with dysentery, were grouped in the genus *Shigella*. So-called species (*S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*) cannot be easily identified by biochemical properties only. Definite identification requires serotyping. There are 15 serotypes in *S. dysenteriae*, 6 serotypes in *S. flexneri*, 18 serotypes in *S. boydii*, and one serotype in *S. sonnei*. Sub-serotypes have been described, and non-agglutinable *Shigella* strains are increasingly isolated. Molecular taxonomy (DNA hybridization) and multiple gene sequence data indicate that all *Shigella* species and serotypes (except *S. boydii* serotype 13) constitute a single genomic species with *E. coli*. *Shigella* should be considered as special *E. coli* clones that are non-motile, anaerogenic, auxotrophic, carrying an invasive plasmid and strictly adapted to humans.

The methods for processing of stools and environmental and food specimens for the detection of *Shigella* differ because of different bacterial loads and the presence of different types of competitive organisms. Ideally, bacteriologic analysis of specimens from patients should be performed within 2-4 hours of collection of samples. If specimens need to be stored, they should be placed at 4 °C in transport media (buffered glycerol in saline or Cary-Blair). Enrichment is not usually required for stool specimens, although enrichment of food and environmental samples may be necessary due to the low numbers of viable organisms present. At least two differential media are normally used for direct plating from stools and can also be used for plating from enrichment broths. One of the two media is usually selective, while the other one is a non-inhibitory medium, such as MacConkey agar. Detection of viable, but non-culturable forms in environmental samples is best achieved by polymerase chain reaction (PCR) or immu-

noassays. Immunomagnetic separation can be used for the enrichment of bacteria from stools to increase the sensitivity of detection. Real time PCR is the most rapid detection method but is expensive.

Nine thousand and two hundred strains of *Shigella* species isolated from patients at ICDDR,B during January 1997–December 2003 were analyzed. *S. flexneri* (60%) was the most predominant species, followed by *S. boydii* (17%), *S. dysenteriae* (12%), *S. sonnei* (8%), and *Shigella*-like organism (3%). The incidence of atypical strains of *Shigella* is rising and accounts for 23% of *S. flexneri*, 8% of *S. dysenteriae*, and 23% of *S. boydii* strains. Overall, the pattern of prevalence of various serotypes of *S. flexneri* is changing with the emergence of new/atypical serotypes within *Shigella* spp. in Bangladesh and Pakistan. More than 500 strains of *S. dysenteriae* type 1 were examined using PFGE (pulsed-field gel electrophoresis). Changes in PFGE type (clone C) were recently observed, and a single clone (C1) is disseminating throughout South Asia, reflecting a possible epidemic by this clone in the future. There is an urgent need to produce commercial antibodies against these serotypes to recognize the contribution of these atypical *Shigella*-like strains in the global burden of shigellosis.

PATHOPHYSIOLOGY AND CLINICAL MANAGEMENT OF SHIGELLOSIS

The use of antimicrobials in shigellosis alleviates the dysenteric syndrome, fever, and abdominal cramps, reduces the duration of pathogen excretion, interrupts transmission of disease, and reduces the risk of potential complications. However, empirical antimicrobial therapy requires the knowledge of the antimicrobial resistance pattern of *Shigella* strains circulating locally. The trends in antimicrobial resistance pattern were reviewed from the published literature and using data from stool or rectal swab cultures from hospital patients and outpatients (1970-2003) and from community-based surveillance in an urban slum of Dhaka city (2002-2003). The choice of antimicrobials in treating shigellosis is now limited. Tetracycline, ampicillin, cotrimoxazole, and nalidixic acid are no longer effective in many countries, especially in patients infected with *S. dysenteriae* type 1. The antimicrobials that remain effective are mecillinam, ciprofloxacin, other fluoroquinolones, ceftriaxone, and azithromycin. However, in 2003 and 2004, a few cases of ciprofloxacin- and other fluoroquinolones-resistant *S. dysenteriae* type 1-

associated infection have been reported from India, Bangladesh, and Nepal.

In the wake of an alarming increase in antibiotic resistance in *Shigella* spp., alternative medicines are an attractive approach. Antibacterial peptides, such as LL-37, are the first line of host defense that prevents microbial intruders. Sodium butyrate, a short-chain fatty acid normally produced by the gut flora, was shown to induce up-regulation of this peptide in the colonic cell lines. In the rabbit model of shigellosis, butyrate treatment was shown to enhance expression of CAP-18, the precursor of LL-37, in the surface epithelial cells and reduction in inflammation, reduction in bacterial count in stool, and improved clinical recovery. In addition, supplementing protein-rich diet during convalescence significantly enhances catch-up growth, while prolonged supplementation of zinc has both therapeutic and preventive impacts on diarrhoeal illnesses. Nevertheless, further studies are needed to see whether zinc and butyrate supplementation can be used as adjuvants in the treatment of multidrug-resistant *Shigella*-associated infection.

Complications of shigellosis are multiple. The commoner complications of shigellosis include: stress ulcer, necrotizing enterocolitis, third-degree rectal prolapse, reactive arthritis, and less commonly Reiter's syndrome and polyarthritis. The more common and more severe, often life-threatening, complications of shigellosis include: hyponatraemia, hypoglycaemia, bacteraemia and septicaemia, leukaemoid reaction, haemolysis, and acute renal failure, including haemolytic-uraemic syndrome (HUS), intestinal obstruction/toxic megacolon, various types of central nervous system (CNS) disorders, and negative acute and long-term nutritional impact. Rare complications include: urinary tract infections and prostatitis; intestinal perforation, appendicitis, and splenic abscesses; in-utero foetal transmission; rhabdomyolysis, joint infections, and osteomyelitis; pneumonia; and cardiac (myocarditis, bradycardia, A-V block), ocular (conjunctivitis, corneal ulcer, uveitis, etc.), hepatic (cholestasis, hepatic failure), cutaneous (purpura, urticaria, etc.) and nervous system (central speech disorder, peripheral neuropathy, Guillain-Barre syndrome, etc.) complications.

With the limited data available at present from sub-Saharan Africa, there is no suggestion of a significant interaction whereby HIV predisposes infected adults or children to infection with shigellosis. Furthermore, there

is no evidence of increased resistance patterns to strains of *Shigella* as a consequence of the HIV epidemic.

CONCLUSION

The review of the situations with regard to shigellosis led to the revision of the WHO guidelines for the control of dysentery. The main modifications brought to these guidelines concerned the following:

- The development of a guideline for the control of dysentery in general rather than focused on outbreaks due to *S. dysenteriae* type 1 only
- The need to put more emphasis on interventions targeting, among other prevention methods, hand-washing practices
- The need to change antibiotics recommended for the treatment for shigellosis. Nalidixic acid should not be recommended anymore. Fluoroquinolones should be used as first-line treatment for all patients suffering from shigellosis. Patients should also receive supplementation of zinc.

A modified version of the WHO guidelines will be prepared and available in the near future.

In addition, a number of research topics to be addressed in priority were listed. These are as follows:

- Research to improve interventions dedicated to hand-washing practices
- Research on surveillance and risk factors for outbreaks
- In the domain of case management, research on antimicrobials should be the priority. This ranges from developing new drugs, adapting treatment regimens, developing combination therapy, to new packaging and presentation
- The role of non-antimicrobial agents, diet, and vitamin A should also be further studied
- The development of reliable rapid diagnostic assays and of good enrichment medium should urgently be addressed for improving diagnosis and surveillance, and research on *Shigella* in the environment should be conducted. Development and validation of methods to transport DNA versus intact strains is also needed in view of the difficulties in transporting bacterial strains.