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Shigella: A Highly Virulent and Elusive Pathogen

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Abstract Despite a significant decrease in *Shigella*-related mortality, shigellosis continues to carry a significant burden of disease worldwide, particularly in Asia and Africa. *Shigella* is a highly virulent pathogen comprised of four major species with numerous subtypes. *Shigella dysenteriae* and *Shigella flexneri* infections are predominant in resource-limited settings. Clinical presentations range from mild watery diarrhea to severe dysentery with systemic complications such as electrolyte imbalance, seizures and hemolytic uremic syndrome. *S. dysenteriae* subtype 1, the producer of Shiga toxin, causes the most severe illness and highest mortality. Susceptible strains of *Shigella* may be effectively treated with inexpensive oral antibiotics such as ampicillin or trimethoprim-sulfamethoxazole. Unfortunately, multidrug-resistant strains have emerged that have rendered most antibiotics, including fluoroquinolones and extended-spectrum cephalosporins, ineffective. Management and prevention of shigellosis represents a major public health challenge. The development of an effective vaccine is urgently needed to decrease its global impact.

Keywords *Shigella* · Morbidity · Mortality · Antimicrobial resistance · Epidemiology · Socioeconomic conditions · Virulence factors · Vaccine · Developing countries · Industrialized countries · Type III secretion system · Serotype switching · Immune evasion · Fluoroquinolones · Extended-spectrum cephalosporins · Ipa · Tropical medicine · Bacterial

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Introduction

While child mortality has markedly decreased in recent decades, diarrheal disease remains the second leading cause of mortality among children under five years of age [1]. *Shigella,* a highly virulent pathogen that causes bacterial dysentery, is one of the leading causes of diarrheal disease and contributes significantly to the burden worldwide. Despite numerous efforts directed at its prevention and control, that goal remains elusive. This paper will discuss important features of the epidemiology, pathogenesis, and clinical presentation of shigellosis and highlight recent trends and developments relevant to its prevention and management.

Shigella- Related Mortality, but not Morbidity, has Decreased Substantially Over the Last 50 Years

Shigellosis has undergone significant changes over recent decades. In 1999, Kotloff et al. [2] estimated that~165 million cases of shigellosis and 1.1 million shigellosis-related deaths, most of which occurred in Asia, occurred annually between 1966 and 1997. However, a recent review for the 1990 to 2009 time period [3] found that while the total number of shigellosis episodes in Asia had negligibly decreased, the total number of shigellosis-related deaths substantially decreased to approximately 14,000 per year. The authors proposed that the decrease in *Shigella*-related deaths could be a consequence of the widespread implementation of non-specific interventions such as measles vaccination, vitamin A supplementation, and improved nutrition. These results concur with a comprehensive study from China [4] that found a 3-fold decrease in morbidity and a 10-fold to 31-fold decrease in mortality from bacillary dysentery in China from 1991 to 2000. Another

factor that may have contributed to a worldwide reduction in *Shigella*-related deaths could be the still-unexplained decrease in *Shigella dysenteriae* type 1 (SD1) over the last decades. This serotype, a cause of large-scale dysentery outbreaks and high case fatality rates, was widespread in Latin America, Asia, and Africa two to four decades ago [5–8]. Currently, SD1 infections are primarily reported on the Indian subcontinent, although outbreaks and sporadic infections are also reported in Africa [9–12].

Despite the substantial decrease in mortality, the global burden of shigellosis remains considerable. In a large prospective case-control study in Africa and Asia [13], *Shigella* was isolated from 17 %, 66 % and 78 %, respectively, of infants, toddlers, and children with moderate-to-severe dysentery, and was among the top four pathogens associated with moderate-to-severe diarrhea at all study sites. A large multicenter study in six Asian countries [14] found an overall incidence of 2.1 episodes/1000 residents/year for all ages. Incidence rates were highest in children less than five years of age (13.2 episodes/1000/y) followed by those 70 years and over (2 episodes/1000/y). The incidence of shigellosis was highest in children from Bangladesh (48.2/1000/y) and lowest in children from Thailand (4.0/1000/y). Shigellosis is prevalent in Latin American countries as well. Kosek [15] reported an 8.3 % isolation rate in children with diarrhea from the Peruvian Amazon, while our group isolated *Shigella* from 4.5 % of infants less than three years of age from a rural community in Yucatan, Mexico [16]. A higher prevalence of *Shigella*-associated diarrhea has been reported in hospital settings. Two hospital-based studies in Brazil isolated *Shigella* in 10 % of diarrheal stools from children [17, 18]. During the last three years we have witnessed an upsurge of severe shigellosis at our hospital center in Mexico where it is the cause of acute diarrheal episodes in~11 % of children [19•].

Shigella is a Highly Virulent Organism

Shigella is a gram-negative intracellular bacterial pathogen that initiates infection by invading cells and causing intense inflammation in the colonic and rectal epithelium. A low infective dose on the order of 10 to 100 organisms is sufficient to produce disease. It is typically transmitted by contaminated food and water or by direct contact with an infected person. *Shigella* species possess a large virulence plasmid that carries the genes necessary for cell invasion, including those for a type III secretion system (TTSS) used to gain entry into epithelial cells [20, 21]. The *ipa–ipg* operon encodes for important effector/translocator proteins (IpaA through IpaD) that are injected into the host cell by the TTSS. Once *Shigella* enters the epithelial cell, it escapes from the phagocyte vacuole and actively proliferates within the cytosol of infected cells (Fig. 1) [22, 23]. Recently, Paciello et al. [24•] showed that *Shigella* modifies its membrane lipopolysaccharide (LPS) composition in order to evade pathogen recognition and eradication processes.

In addition to these virulence factors, *Shigella* also may produce one or more of several toxins. Two enterotoxins that cause fluid secretion in animal models [25] have been identified: *Shigella* enterotoxin 1 (ShET-1) and *Shigella* enterotoxin 2 (ShET-2). ShET1, predominantly found in *S. flexneri*, is encoded by the chromosomal gene *set.* It is a classical AB toxin comprised of several B subunits that bind to specific molecules on the target cell and a single A subunit that carries out the toxic enzymatic reaction within the cell. ShET-2 is encoded by a plasmid-borne gene *sen* and may be expressed by all *Shigella* species [26, 27]. Aside from an enterotoxic activity similar to that of ShET-1, ShET-2 is also believed to induce inflammation of epithelial cells via IL-8 secretion [28].

Shiga toxin 1(Stx 1), the most widely known *Shigella* toxin, is produced by SD1. Responsible for the most severe manifestations of shigellosis, Stx1 has an AB5 subunit structure. The five B subunits bind Shiga toxin to the glycolipid Gb_3 receptor present on target cells such as intestinal villi, glomerular endothelial cells, mesangial cells, podocytes, and renal tubular cells [29]. The A subunit is a cytotoxic protein that acts on the 28S rRNA component of eukaryotic ribosomes, leading to protein synthesis inhibition and destruction of endothelial cells [30]. It is also believed to increase the expression of chemokines and cytokines, which in turn leads to chemoattraction and activation of neutrophils, and ultimately, to the binding of inflammatory cells to the endothelium [31].

Shigella Species Shift with Changing Socioeconomic Conditions

Shigella is comprised of four major species: *S. dysenteriae, Shigella flexneri, Shigella boydii*, and *Shigella sonnei.* With the exception of *S. sonnei,* each species is further subdivided into serotypes. *S. dysenteriae* has 15; *S. flexneri* has six, and *S. boydii* has 18 serotypes [32] that are based on the structure of exposed terminal O polysaccharides that form part of their outer membrane LPS [33]. Several investigators have noted an association between *Shigella* species and socioeconomic conditions [34•, 35]. *S. dysenteriae* and *S. flexneri*, associated with poverty and poor hygiene, are the predominant species in resource-limited settings, while *S. sonnei* is more common in affluent regions [35–37]. Presently, SD1 is endemic and epidemic on the Indian subcontinent [9, 10, 38], while *S. flexneri* is predominant throughout Southeast Asia [14, 34•, 39, 40], Latin America [15, 17, 19•], and Africa [12, 41]. A shift in the dominant infecting species from *S. flexneri* to *S. sonnei* has been documented in countries that have undergone recent improvements in economic status such as China, Vietnam, and Thailand [14, 34•, 40]. The persistence

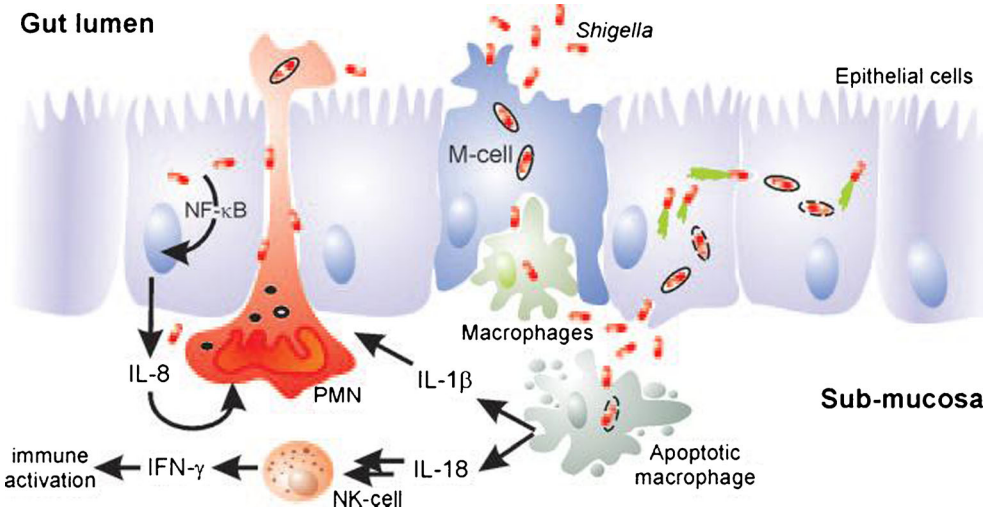

Fig. 1 In the colonic mucosa, *Shigella* is transcytosed across M cells into the underlying gut-associated lymphoid tissues. They later enter macrophages and induce apoptosis, leading to release of the bacteria on the basal side of the epithelium. Upon receipt of a secretion signal, *Shigella* invades epithelial cells from the basolateral side and spreads to adjacent cells. Proinflammatory signaling by host cells further activates the immune response, which initially exacerbates infection and tissue destruction. Ultimately, the infection is resolved when PMN phagocytose and kill the invading *Shigella*. (Reproduced with permission from Schroeder GN, Hilbi H. Molecular Pathogenesis of *Shigella* spp.: Controlling Host Cell Signaling, Invasion, and Death by Type III Secretion Clin. Microbiol. Rev. 2008; 21: 134-156)

of *S. flexneri* in Latin America likely reflects the prevailing poverty in the region. In industrialized countries, *Shigella* infections have frequently been found in day care centers or linked to consumption of imported food [42–44].

Serotype Switching has Important Implications for Vaccine Development

During natural infection, *Shigella* elicits an antibody-mediated, serotype-specific immune response. Serotype switching through O-antigenic variation is a common strategy used by the organism to evade host immunity [45]. The replacement of commonly circulating serotypes by newly emerging ones in response to selective pressure from population immunity has been well documented in China where this phenomenon decreased the effectiveness of a *S. flexneri* 2a-based vaccine [34•, 46].

There is consensus within the scientific community that a global *Shigella* vaccine must protect against SD1, *S. sonnei*, and all *S. flexneri* serotypes [47]. Various candidate vaccines have been developed and tested in clinical trials including killed bacteria, live attenuated strains, and polysaccharide conjugates and LPS-protein mixtures. Due to poor immunogenicity, limited scope, or strong adverse reactions, however, none of these has been entirely satisfactory for large-scale use [47–49]. More recently, IpaB and IpaD, two type-III secretion proteins that are highly immunogenic and conserved across *Shigella* species, have been tested for their use in cross-protective vaccines [50, 51]. Intranasal immunization with a subcellular vaccine prepared from pathogenic *S. flexneri* composed of LPS, IpaB, IpaC and IpaD was well tolerated and induced strong gastrointestinal mucosal IgA responses in 58 % and plasma IgA responses in 50 % of volunteers [50]. Despite these encouraging results, improvement will be necessary to achieve higher conversion rates and demonstrate protective efficacy.

The Clinical Presentation of *Shigella* Infections is Very Variable and Depends on the Infecting Species

Shigellosis is typically an invasive infection of the human colon and rectum, causing severe inflammation and tissue necrosis. Clinical presentations, nonetheless, are highly variable. Some patients have a mild, watery diarrhea with no fever, while others have severe dysentery, high fever, and systemic complications [14, 19•, 40, 52•]. *Shigella* infections may also lead to persistent diarrhea in young children [14]. Although all four *Shigella* species may cause both intestinal and extra-intestinal disease, SD1 is associated with the greatest severity. In Bangladesh [52•], children infected with SD1 more frequently had grossly bloody stools, abdominal tenderness, leukemoid reaction, and rectal prolapse when compared to children infected by *S. dysenteriae* serotypes 2-10 or other *Shigella* species. Seizures have also been reported worldwide [14, 19•, 40, 52•, 53] and are significantly associated with death in impoverished regions [52•]. The rate of seizures is highly variable, especially by region. Extremely high rates have been reported in Thailand (27.5 %) [53]. In Bangladesh [52•], seizures have been documented in 8 % of children with *S. dysenteriae* infections (all serotypes) and 5 %

of *S. flexneri* infections, but in none of the infections caused by *S. sonnei* and *S. boydii.* In Mexico [19•], 11 % of our hospitalized children with *Shigella* gastroenteritis presents with seizures, almost invariably caused by *S. flexneri*. Although most seizures occur with *S. dysenteriae* and *S. flexneri* infections, seizures and severe disease manifestations have also been reported for *S. sonnei* [40, 54•]. In a 14-year study conducted at two large hospitals in Vietnam, Vinh et al. [40] found that 7.3 % of children infected with *S. flexneri* developed seizures compared to 14.3 % of those infected with *S. sonnei*.

Hemolytic uremic syndrome (HUS), comprised of the triad of hemolytic anemia, thrombocytopenia, and renal insufficiency, occurs in about 8-13 % of dysenteric patients infected with SD1, with a case fatality rate of 36 %. It may occur, albeit rarely, with *S. flexneri* infections [55, 56]. SD1 HUS typically develops one week or more after the onset of diarrhea. In contrast to *E. coli* O157:H7, it occurs after a course of bloody diarrhea with fever, almost exclusively in young children. Primarily due to the action of Shiga toxin on microvascular endothelial cells, most damage occurs in the kidneys and CNS [57]. End-stage renal disease and hypertension have also been reported, although less often than with STEC-related HUS [58]. Rare, but severe, complications include encephalopathy, hemiplegia, cardiomyopathy, and disseminated intravascular coagulation [58].

It should be noted that most published studies have been conducted in hospital settings, which leads to a selection bias towards greater severity. For example, 36 % of children hospitalized for *Shigella* gastroenteritis at our center had a fever greater than 38.5 ° C, compared to only 7 % of young infants in a rural community in Yucatan, Mexico [16, 19•]. Dehydration and seizures were present, respectively, in 28 % and 11 % of the hospitalized children, but in none of the children from the community setting. Notably, the presence of grossly bloody stools was similar (59 % in hospitalized children compared to 47 % in children from the community). In highly endemic settings where the population is repeatedly exposed to *Shigella* and other enteropathogens, protective immunity is acquired in infancy and tends to wane in old age. Susceptibility in elderly people, however, does not return to that of immunologically naïve infants [16].

Increasing Multidrug Resistant *Shigella* is a Serious Public Health Threat

Shigella can cause severe illness and persistent diarrhea; young infants, the elderly, and immunosuppressed individuals are at particular risk and require prompt antimicrobial treatment. *Shigella* dysentery may be effectively treated with inexpensive oral antibiotics such as ampicillin or trimethoprim-sulfamethoxazole when strains are susceptible. Unfortunately, our capacity to effectively treat severe infections is being undermined by increasing resistance to antimicrobial compounds worldwide. At present, countries with the greatest *Shigella* burden also face greater challenges of multidrug resistance. A recent review [59•] of antimicrobial resistance trends worldwide showed that quinolone resistance in the Asia-Africa region increased at a distressingly rapid rate over the last decade, and that the proportion of resistant strains is more than ten times the rate in Europe and America. Worldwide, strains are becoming resistant not only to first-line oral antibiotics such as ampicillin, trimethoprim-sulfamethoxazole, and tetracycline, but to broad-spectrum antibiotics as well [14, 40, 60]. Outbreaks of multidrug-resistant, fluoroquinolone-resistant SD1 strains have occurred in India and Bangladesh over the last decade [9, 10, 38], and fluoroquinolone-resistant *S. flexneri* has been reported from China [46, 59•]. Ceftriaxone-resistant *S. flexneri* and *S. sonnei* have been reported from south Asia [40, 46, 59•, 61] and the Middle East [62, 63]. In Latin America, most strains remain fully susceptible to fluoroquinolones and third-generation cephalosporins [15, 17, 18, 19•].

Ciprofloxacin, pivmecillinam, and ceftriaxone are currently recommended by the World Health Organization for the treatment of multiresistant *Shigella* strains [32]. Azithromycin is another therapeutic option, although its use has been associated with rapid development of resistance. At our hospital center in Mexico we have used 3-to-5-day regimens of azithromycin in children of all ages with bloody diarrhea; notable clinical improvement has been observed within 24 hours for most patients, even for those strains with MICs in the intermediate range. We strongly recommend that susceptibility to azithromycin be tested by agar or broth dilution, as we have observed discordant results using disk diffusion (authors' unpublished results). For children unable to tolerate oral dosing or those in hypovolemic shock, we have successfully used intravenous or intramuscular ceftriaxone. Oral extended-spectrum cephalosporins are less effective for the treatment of shigellosis; a high rate of clinical failure has been observed in both adults [64] and children [65] treated with cefixime.

SD1 infections merit special mention due to the association of Shigella–associated HUS and previous antimicrobial treatment. In both SD1 and Shiga toxin-producing *Escherichia coli* (STEC), the genes encoding Shiga toxin are chromosomally encoded and linked with lambdoid bacteriophage sequences. In contrast to STEC infections, in which the use of antibiotics induces lysogenesis and increased Shiga toxin expression leads to increased mortality, the prophage in SD1 strains is defective and unable to induce lysogenesis [66]. Although exposure of SD1 to antibiotics does not increase Shiga toxin expression via bacteriophage lysogenesis, extracellular concentrations may increase after antibiotic-mediated killing and bacterial lysis [67].

Previous studies reported an increased association between use of trimethoprim-sulfamethoxazole and ampicillin and subsequent development of HUS [55]. Lower rates of HUS were observed in patients who received ciprofloxacin, nalidixic

acid, and ceftriaxone, although the poorer outcomes are believed to be related to clinical failures due to antibiotic resistance [55, 67]. Data from clinical trials has shown that if antimicrobial therapy is administered within the first three days after onset of dysentery, the incidence of HUS is very low. Bennish et al. [67] analyzed the data for 378 individuals infected with SD1 who received antimicrobial treatment before 96 hours after the onset of symptoms, of which 93 % received an antimicrobial agent to which the organism was susceptible. The risk of developing HUS was 0.0026 for all participants and 0.004 for children.

Conclusions

Despite numerous efforts at prevention and control, shigellosis continues to be an important cause of acute diarrhea and dysentery worldwide. Factors that contribute to the persistence of shigellosis worldwide are poor socioeconomic conditions, serotype switching, and increasing antimicrobial resistance. Major challenges for the future are the development of an effective vaccine that can protect against strains that produce clinically important disease and the prevention and containment of multidrug-resistant *Shigella* strains. The Ipa effector proteins are promising candidates for a subunit-based vaccine. Greater efforts are required to further contain the widespread resistance to fluoroquinolones and extended-spectrum cephalosporins.

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Compliance with Ethics Guidelines

Conflict of Interest Mussaret Bano Zaidi and Teresa Estrada-García declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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