Infectious diseases kill about 11 million children each year while acute diarrhoeal diseases account for 3.1 million deaths in children under 5 yr of age, of which 6,00,000 deaths annually are contributed by shigellosis alone. Shigellosis, also known as acute bacillary dysentery, is characterized by the passage of loose stools mixed with blood and mucus and accompanied by fever, abdominal cramps and tenesmus. It may be associated with a number of complications of which haemolytic uraemic syndrome is the most serious. Shigellosis is caused by Shigella spp. which can be subdivided into four serogroups namely S. sonnei, S. boydii, S. flexneri and S. dysenteriae. Organisms as low as 10-100 in number can cause the disease. Shigellosis can occur in sporadic, epidemic and pandemic forms. Epidemics have been reported from Central American countries, Bangladesh, Sri Lanka, Maldives, Nepal, Bhutan, Myanmar and from the Indian subcontinent, Vellore, eastern India and Andaman and Nicobar islands. Plasmid profile of shigellae in Kolkata has shown a correlation between presence of smaller plasmids and shigellae serotypes- indicating epidemiological changes of the species. Diagnosis of shigellosis is essentially clinical. Laboratory diagnosis includes stool culture and polymerase chain reaction (PCR). Treatment includes use of an effective antibiotic, rehydration therapy (if there is dehydration) and appropriate feeding during and after an episode of shigellosis. Hand-washing is the single most important strategy for prevention of transmission of shigellosis from person to person. A safe and effective vaccine should be developed against the more important circulating strains i.e., S. dysenteriae type 1 and S. flexneri 2a.

Key words Drug resistance - dysentery - shigellae - shigellosis

Infectious diseases kill about 11 million children each year and 99 per cent of these deaths occur in the developing countries. Notably, of the 11 million deaths, 4 million die within the first year of their life. Acute diarrhoeal diseases rank second amongst all deaths due to infectious diseases accounting for 3.1 million deaths in under 5 children; 80 per cent of these deaths occur in children below 2 yr of age. Shigellosis is an important cause of diarrhoeal deaths. It has been reported that no less than 140 million cases of shigellosis occur worldwide with 600,000 deaths annually; 60 per cent of such deaths are seen in under 5 children. In this review, attempts have been made to highlight the molecular epidemiology, epidemic and pandemic potential, current case management strategies including drug resistance problem and preventive aspects of shigellosis.

Definition

Shigellosis, commonly known as acute bacillary dysentery, is manifested by the passage of loose stools mixed with blood and mucous and accompanied by fever, abdominal cramps and tenesmus (a symptom characterized by incomplete sense of evacuation with rectal pain).
Clinical features

In some cases, there may not be any symptoms (asymptomatic), while in others it may produce mild to moderate dysentery or even fulminating dysentery with fever, severe abdominal cramps and rectal pain. Children may have high fever (104°F) with convulsions, rectal prolapse and later develop malnutrition. *Shigella sonnei* produces mild dysentery. *S.flexneri* and *S.dysenteriae* type 1 typically produce severe dysentery, particularly the latter.

Complications

Shigellosis may be associated with a large number of mild to severe life-threatening complications, particularly due to *S.dysenteriae* type 1. Children may have high fever, rectal prolapse and convulsions. Arthritis and arthralgia are complained by some patients. Intestinal perforation, haemorrhage, toxic megacolon and protein loosing enteropathy may complicate a shigellosis case. Leukemoid reaction (WBC count > 50,000/cmm) and haemolytic uraemic syndrome (a triad of microangiopathic haemolytic anaemia, thrombocytopenia and renal failure) are seen in *S.dysenteriae* type 1 infection and may be fatal.

Epidemiology

Shigellosis is a highly contagious disease caused by *Shigella* spp. and humans are the principal reservoir of infection. The organism is acid resistant and can easily pass the gastric acid barrier. The infective dose is as low as 10-100 organisms only. The disease is transmitted faeco-orally, the commonest modes being person-to-person contact and contaminated food and water. Infected food handlers can spread the disease. Sometimes consumption of raw vegetables harvested in fields where sewage is used as fertilizer can cause contamination. Flies can breed in infected faeces and contaminate food. Washing clothes and bathing in ponds, which is a common practice in rural India, can also enhance transmission of the disease if the water is contaminated with excreta of an infected person. It is a disease of overcrowding, insanitary conditions and poor personal hygiene, and affects mostly children of developing countries. However, travelers visiting endemic areas may be affected by this disease if they do not take proper precautionary hygienic measures.

Incubation period of the disease is 1-4 days which is usually followed by sudden onset of acute symptoms. In mild cases the disease may be self limiting but severe disease requires appropriate medication. The disease is communicable as long as an infected person excretes the organisms in the stool and this can extend up to 4 wk from the onset of illness. However, timely antibiotic therapy can reduce the period of communicability. Secondary attack rates can be as high as 40 per cent especially among household contacts.

Epidemics and pandemics

During 1967-70, bacillary dysentery was first reported in Central American countries. Since then, spread of this infection has been reported from many Asian countries such as Bangladesh (1972-78, 2003), Sri Lanka (1976), Maldives (1982), Nepal (1984-85), Bhutan (1984-85) and Myanmar (1984-85). In India, epidemics were mainly encountered in southern India (Vellore - 1972-73, 1997-2001), eastern India (1984) and Andaman and Nicobar islands (1986). Recent outbreaks (2002-03) of multi drug resistant *S.dysenteriae* type 1 have been reported from Siliguri, Diamond Harbour, Kolkata, and Aizwal and Bangladesh.

Microbiology

Shigellosis is caused by *Shigella* spp. which can be subdivided into four serogroups - *S.sonnei*, *S.boydii*, *S.flexneri* and *S.dysenteriae*. Each of them has a number of serotypes, e.g., *S.dysenteriae* type 1-12, *S.sonnei* phase I and II, *S.boydii* type 1-18, *S.flexneri* type 1-6. However, three predominant strains are responsible for majority of shigellosis cases viz., *S.sonnei*, *S.flexneri* 2a and *S.dysenteriae*.
type 1. Of these, *S.*sonnei is encountered mostly in industrialized countries, *S.*flexneri 2a in developing countries and *S.*dysenteriae type 1 is the only epidemic as well as pandemic strain. *S.*dysenteriae type 1, which produces severe disease, may cause life-threatening complications, is usually multi drug resistant and can cause large epidemics and even pandemics with high morbidity and mortality.

Shigellae are facultative non motile, Gram negative bacilli. They are pathogenic primarily due to their ability to invade intestinal epithelial cells. The virulence factor is a smooth lipopolysaccharide cell wall antigen which is responsible for the invasive features and a toxin (shiga toxin) which is both cytotoxic and neurototoxic and causes watery diarrhoea.

Shigella organisms invade and multiply within the colonic epithelial cells causing cell death and mucosal ulcers but rarely invade the blood stream. Histological findings include cellular infiltration with mixed round cells, neutrophilic in majority and disorganization of crypts with branching and dilatation. The inflammatory process extends to muscularis mucosae and submucosae with resultant oedema. In *S.*dysenteriae type 1 infection, changes in the colonic mucosa are more severe than those by the other serogroups.

Drug resistance

When sulphonamides were first introduced in the early 1940s, all the *Shigella* strains were sensitive to this drug, which became the drug of first choice. In late 1940s, tetracycline followed by chloramphenicol, were recommended for the treatment of shigellosis because sulphonamides became ineffective. Soon, resistance to these two drugs was observed. Ampicillin and co-trimoxazole came to the rescue. They were found to be clinically highly effective. However, during the epidemic in eastern India in the late 1980s, the isolated *S.*dysenteriae type 1 strains were found to be resistant to most of the antibiotics except nalidixic acid which was found to be clinically highly effective. But later, *S.* dysenteriae type 1 strains isolated from an outbreak in Tripura were even resistant to nalidixic acid. In the late 1980s, fluoroquinolones (norfloxacin, ciprofloxacin and ofloxacin) were introduced and were found to be very effective in the treatment of shigellosis cases including those caused by multi drug resistant *S.*dysenteriae type 1 strain. Recent outbreak investigations in India (Siliguri, Diamond Harbour, Kolkata, and Aizwal) and Bangladesh showed high level of resistance even to norfloxacin, ciprofloxacin and ofloxacin. Only ceftrioxone and azithromycin are now clinically effective for the treatment of multi drug resistant shigellosis. However, ceftrioxone has to be administered parenterally and is expensive. In Bangladesh, pivmecillinum has been found to be useful.

Mechanisms of antibiotic resistance

Treatment of shigellosis has been confounded by wide spread resistance to the commonly used antibiotics such as ampicillin, co-trimoxazole, tetracycline, nalidixic acid and recently to norfloxacin and ciprofloxacin. The transmissibility of resistance can take place by clonal spread of particular strains as observed in *S.* dysenteriae type 1. Horizontal transfer of resistance determinants by plasmids, transposon-mediated conjucation and/or chromosome also occurs. Presence of integrons in shigella with multi drug resistant genes and the ability to spread in epidemic form necessitates intervention at the public health level. Studies conducted with *S.* sonnei isolated in Ireland showed the prevalence of class 1 and class 2 integrons carrying aadA cassettes along with sat1 and dhfr1. In Australia, it was shown that most of the *S.* sonnei strains harboured class 2 integron with a gene cassette array analogous to that found in transposon (Tn7), namely dfrA1, sat1, and aadA1 conferring resistance to trimethoprim, streptothricin and streptomycin/spectinomycin, respectively. These antibiotics were introduced in the 1950s as alternative treatment for sulfonamide resistant *Shigella* and were extensively used in Australia and Ireland. *S.* sonnei strains readily acquire resistance to ampicillin through conjugative resistance-plasmids carrying the resistance cassettes blaTEM-1 and blaTEM-52.

Tetracyclines have been used extensively since the late 1940s as broad spectrum inexpensive antibiotics. However, resistance to tetracycline has increased
dramatically since the first appearance of resistance in 1953 in *S. dysenteriae*\(^{41}\). Most tetracycline resistance determinants, defined as genetic units, which contain both structural and regulatory genes, involved in resistance have been found on resistance plasmids or transposons, making gene transfer the likely method of acquiring resistance. An investigation carried out using about 600 strains collected from six countries demonstrated that the tetracycline resistance in *Shigella* is due to both clonal spread and horizontal gene transfer\(^{42}\).

Quinolones were highly effective drug for the treatment of shigellosis. Resistance to this group of drugs by *Shigella* spp is essentially due to chromosomal mutations\(^{43,44}\). Mutations that confer quinolone resistance principally affect the DNA gyrase and topoisomerase IV of type II. Both the enzymes are heterotetramers consisting of two types of subunits GyrA and GyrB for DNA gyrase and ParC and ParE for topoisomerase IV. DNA gyrase is primarily engaged in the control of negative supercoiling of DNA and topoisomerase is involved in the decatenation of the interlinked daughter chromosome. The mutation recorded due to quinolone resistance was Ser83 to Leu in *S. dysenteriae* type 1, *S. flexneri* and *S. sonnei*\(^{40,43,44}\).

**Plasmid profile**

Multiple antibiotic resistant *S. dysenteriae* type 1 isolates from the 1984 epidemic in Eastern India showed identical plasmid patterns\(^{45}\). During the period 1995-2000, the plasmid profile of shigella strains isolated in Kolkata, revealed the presence of large plasmid of 220kb in majority of the strains (except *S. sonnei*) and there was a correlation between the presence of smaller plasmids and *Shigellae* serotypes – indicating epidemiological changes of *Shigellae* species in Kolkata, India\(^{46}\).

**Clonality of recent multidrug resistant strains of *S. dysenteriae* type 1**

Outbreaks of multi drug resistant *S. dysenteriae* type 1 occurred in several parts of eastern India including northern and southern parts of West Bengal in 2002 and Mizoram in 2003\(^{47}\) and also from Matlab, Bangladesh\(^{9}\). Sporadic cases were also admitted to the Infectious Diseases Hospital and Dr B.C.Roy Children’s Hospital, Kolkata during the same time. Antimicrobial resistance pattern, plasmid DNA and pulsed field gel electrophoresis (PFGE) profiles of both epidemic and sporadic cases revealed their genetic similarity. Chronologically the new clone was detected first during 2002 from epidemic cases followed by sporadic cases in Kolkata and later from Mizoram. The recently emerged *S. dysenteriae* type 1 strains are different from the previous outbreak strains isolated during 1988\(^{48}\).

**Diagnosis**

Diagnosis of shigelllosis is made clinically by the typical features of bacillary dysentery with blood and mucus in stool although some cases may present with mild to moderate watery diarrhea initially. Dehydration is usually not a conspicuous feature. Microscopic examination of faecal smear stained with iodine shows presence of plenty of faecal leucocytes (> 10/high power field). Confirmation is made by stool culture, serological and biochemical tests\(^{49}\).

**Collection, transportation and culture of stool specimen**

Specific diagnosis of shigella in stool specimens depends on the appropriate collection and transportation to the laboratory. Fresh stool samples collected from patients before initiation of therapy are preferred for microbiological tests because the chances of recovering the organisms are higher. For microbiologic cultures, fresh stool is preferred to rectal swabs in which the pathogens are less in number. Samples that cannot be cultured immediately should be kept in buffered glycerol-saline transport medium. Cary-Blair medium is the second option. Direct inoculation of culture plates at the bedside is the most efficient means of isolating shigella from the dysentery patients.

Stool specimens for isolation of shigella should be plated on both moderately selective medium such as MacConkey or deoxycholate citrate agar (DCA), and a highly selective medium such as xylose-lysine...
deoxycholate (XLD), Hektoen enteric (HE) or Salmonella-Shigella (SS) agar. Since the Shigella isolates growing in these plates do not change the colour of the pH indicator due to its inability to ferment lactose, it is easy to pick up the typical colonies.

Further identification can be made by using triple sugar iron (TSI) agar or Kligler iron agar (KIA), on which Shigellae are non-motile, produce an alkaline slant and acid butt due to inability to ferment lactose aerobically in the slope and the anaerobic fermentation of glucose in the butt, and fail to produce hydrogen sulphide or other gas. After tentative identification, strains can be speciated by serological methods, using grouping antisera. Rapid methods for the diagnosis of S. dysenteriae type 1 by means of fluorescent antibody staining have been established50. Till date, no reliable rapid method is commercially available and none are in use routinely anywhere.

Detection using polymerase chain reaction (PCR)

Most of the PCR methods established for the identification of shigella are targeted towards either invasive-associated locus (ial) gene or invasive plasmid antigen (ipa) H locus, which are also present in the enteroinvasive Escherichia coli (EIEC)51-55. An immunocapture PCR was established which employs amplification to detect bacteria captured by specific antibodies coupled either to the beads or polystyrene plates56,57. The use of IS630-specific primers along with serotype specific primers derived from the rfc genes in the multiplex PCR was reported to be useful for the detection of many serotypes of Shigella58.

In most of these studies, PCR was found to be more sensitive and specific technique than the conventional culture methods and has the potential to be employed in routine diagnosis. In addition, in most of the Shigella strains there is a spontaneous loss of the virulence genes, and hence direct stool PCR based detection system is preferred than the DNA probe hybridization technique in which the strains should be cultured several times.

Management

Diagnosis of shigellosis can be made clinically by the typical features of the disease. Sometimes laboratory confirmation is necessary especially for antimicrobial resistance pattern as it varies from place to place and from time to time.

In case management of shigellosis antibiotics play a central role. Use of appropriate antibiotic hastens recovery, shortens the duration of excretion of pathogen in stool and possibly prevents complications. However, these should be chosen carefully considering the sensitivity pattern of the circulating strains. Presently in India, the antibiotics of choice are norfloxacin (400 mg) or ciprofloxacin (500 mg) or ofloxacin (200 mg) twice daily for 3-5 days59. These drugs are not yet recommended for children and pregnant women although their use has shown that they are probably safe in children60.

Although dehydration is not a common feature of shigellosis infection, but if it occurs or the stools are watery, patients should be given the oral rehydration salt (ORS) recommended by WHO /UNICEF. In severe dehydration, intravenous fluids preferably Ringer’s lactate solution is recommended. However, clinical experience indicates that ORS is beneficial in all cases of shigellosis if given as routine fluid intake.

Anorexia poses a major problem for feeding especially in children. They should be encouraged to take small, frequent and easily digestible meals. This is easily achieved after an effective antibiotic is started when appetite improves and the patient is able to take food.

Prevention and control

Since the main route of transmission of shigellosis is through water, food and also person-to-person contact, the prevention and control strategies essentially include provision of safe water supply and adequate sanitation facilities, maintenance of good personal hygiene and food safety. Hand washing with plenty of water and soap is the most important single effective preventive strategy against shigellosis61. It is emphasized that hands should be washed before eating, before feeding children, after defaecation and after disposal of children’s excreta. These measures
are further reinforced in epidemic situations, when because of the very low infective dose of the organism and its potential for rapid spread, stringent control measures need to be instituted through simple but effective health education messages to the common masses.

Vaccines

In view of the difficulties in implementing the preventive and control measures, the high prevalence of multi drug resistant \textit{S.dysenteriae} type 1 strains and their propensity to cause large epidemics and even pandemics, development of safe and effective vaccines against \textit{S.dysenteriae} type 1 and \textit{S. flexneri} 2a is an attractive proposition. WHO has given high priority for shigella vaccine development programme.

Protection against clinical infection is the rationale of a shigella vaccine. However, interference with establishment of infection and colonization is also beneficial. Since shigellosis can be caused by at least three predominant strains, a trivalent vaccine would be most effective. Presently several candidate vaccines are in the pipeline including a parenteral conjugate vaccine consisting of \textit{S.sonnei} detoxified lipopolysaccharide (LPS) linked to a \textit{Pseudomonas aeruginosa} carrier protein and a nasally administered shigella-proteosome vaccine consisting of shigella LPS non-covalently linked to protein of group B \textit{Neisseria meningitides}. Among other potential vaccines, a combination of live attenuated \textit{S.flexneri} 2a strain CVD 1207 and \textit{S.dysenteriae} type 1 strain CVD 1253 has proved to be safe and immunogenic. Another live attenuated \textit{S.flexneri} 2a strain SC 602 vaccine has successfully undergone phase I and II trials in the USA and Bangladesh.\textsuperscript{62}

Conclusion

Shigellosis is an important public health problem with high morbidity and also mortality mainly among children in developing country situations where overcrowding and poor personal hygiene are rampant. Of all the \textit{Shigellae} spp., \textit{S. dysenteriae} type 1 is notorious for producing not only large scale epidemics but also pandemics which are characterized by multiple drug resistance and several serious complications including haemolytic uraemic syndrome (HUS). The mainstay of treatment is appropriate antibiotics but development of drug resistance poses a serious therapeutic challenge. Preventive long-term measures like improved sanitation and personal hygiene may be a difficult task to achieve in the near future specially among the impoverished urban and rural communities. Thus, an alternative preventive strategy in the form of suitable vaccines against \textit{S.dysenteriae} type 1 and \textit{S.flexneri} 2a is urgently required to save mankind from the scourge of this dreaded disease.

References


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