Review Article

Indian J Med Res 143, May 2016, pp 565-576
DOI:10.4103/0971-5916.187104

Shigellosis: Epidemiology in India

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Received March 12, 2014

Shigellosis is one of the major causes of diarrhoea in India. The accurate estimates of morbidity and mortality due to shigellosis are lacking, though it is endemic in the country and has been reported to cause many outbreaks. The limited information available indicates *Shigella* to be an important food-borne pathogen in India. *S. flexneri* is the most common species, *S. sonnei* and non-agglutinable shigellae seem to be steadily surfacing, while *S. dysenteriae* has temporarily disappeared from the northern and eastern regions. Antibiotic-resistant strains of different *Shigella* species and serotypes have emerged all over the world. Especially important is the global emergence of multidrug resistant shigellae, notably the increasing resistance to third generation cephalosporins and fluoroquinolones, and also azithromycin. This calls for a continuous and strong surveillance of antibiotic resistance across the country for periodic updation of the local antibiograms. The prevention of shigellosis is desirable as it will substantially reduce the morbidity associated with diarrhoea in the country. Public health measures like provision of safe water and adequate sanitation are of immense importance to reduce the burden of shigellosis, however, the provision of resources to develop such an infrastructure in India is a complex issue and will take time to resolve. Thus, the scientific thrust should be focused towards development of a safe and affordable multivalent vaccine. This review is focused upon the epidemiology, disease burden and the therapeutic challenges of shigellosis in Indian perspective.

Key words Epidemiology - India - MDR *Shigella* - molecular epidemiology - shigellosis

Introduction

Shigella species, members of the family *Enterobacteriaceae*, are responsible for causing acute gastroenteritis which is one of the most common causes of morbidity and mortality in children in developing countries[1]. Shigellosis is ubiquitous in impoverished populations of Asian and African countries and antibiotic-resistant strains of different *Shigella* species and serotypes have emerged all over the world[2]. Although shigellosis is associated with a few medical complications only, adequate control of this disease may reduce the overall diarrhoea burden globally. The diagnosis of shigellosis is made by culture isolation of *Shigella* from faeces or rectal swabs. Antibiotic treatment is usually recommended in patients with moderate or severe symptoms as it can reduce the duration and severity of symptoms, excretion of organisms, and prevent complications[3]. However, empiric antimicrobial therapy requires knowledge of the local antibiogram of circulating

Shigella strains. Especially important is the awareness of the global emergence of multidrug resistant (MDR) shigellae, notably the increasing resistance to third generation cephalosporins and fluoroquinolones, and most recently azithromycin[3-6]. In this review, we will focus upon shigellosis in Indian perspective, mainly addressing the epidemiological parameters, disease burden, molecular epidemiology and the therapeutic challenges of emerging MDR shigellae.

History

Hippocrates used the term dysentery to indicate a condition characterized by the frequent passage of stool containing blood and mucus, accompanied by straining and painful defecation[1]. At the end of the 19thcentury, epidemics of bacillary dysentery occurred periodically in Japan, when Kiyoshi Shiga examined dysenteric stools of patients and isolated a bacterium that was agglutinated by serum from convalescent patients but not with acute disease. Later, to honour Shiga, this bacterium was christened as *Shigella dysenteriae* type 1, the first organism of the genus *Shigella*[7]. Following this discovery, the next few decades witnessed additional groups of related organisms being discovered and placed in this genus and named to honour the lead workers, Flexner, Sonne, and Boyd[8-10].

Classification

The genus *Shigella* belongs to family *Enterobacteriaceae*. It comprises four species, *S. dysenteriae*, *S. flexneri, S. sonnei*, and *S. boydii,* which are further classified into serotypes based on biochemical differences and variations in their O-antigen. Thus, *S. dysenteriae* (group A) has 17 serotypes, *S. flexneri* (group B) has 14 classical serotypes and subserotypes, *S. sonnei* (group C) has a single serotype and *S. boydii* (group D) has 20 serotypes[11].

Serogroup distribution: *S. dysenteriae* usually presents in epidemic and outbreak forms of the disease. *S. flexneri* and *S. sonnei* are mainly responsible for endemic disease in developing and developed nations, respectively, while *S. boydii* is restricted to India and neighbouring countries[12]. *S. flexneri* was found to be the most commonly isolated species (68%) in a multi-centric study from six Asian countries including China, Vietnam, Thailand, Bangladesh, Pakistan, and Indonesia, except in Thailand where *S. sonnei* was the commonest (84%), while *S. dysenteriae*, which is most often seen in southern Asia and sub-Saharan Africa, constituted only 4 per cent of the isolates[2].From India, we have observed temporal shifts in prevalent serogroups of shigellae at our tertiary care referral centre which caters to patients from eight adjoining States (Chandigarh, Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir, western parts of Uttar Pradesh, Uttaranchal, and some parts of Rajasthan) thus representing a large geographical area. *S. flexneri* stood as the most common serogroup from 1994-2002, followed by *S. dysenteriae* type 1 emerging as the predominant serogroup after a decade in 2003. *S. flexneri* again emerged as the predominant serogroup since 2004[4,13-16]. Such cyclical changes have also been reported from National Institute of Cholera and Enteric Disease (NICED), Kolkata, in eastern India where epidemics caused by *S. dysenteriae* periodically occur after a gap of a decade or so[17]. Overall the authors have shown *S. flexneri* (60%) as the most prevalent serogroup, followed by *S. sonnei* (23.8%), *S. dysenteriae* (9.8%) and *S. boydii* (5.7%)[17]. Similarly, from Manipal and Puducherry in south India, *S. flexneri* figures as the predominant serogroup representing >90 per cent of the isolates[18,19]. Interestingly, a lesser prevalence of *S. sonnei* has been reported from these two centres (3.9-5.4%) in south India as compared to eastern India (23.8%)[17-19]. This observation was corroborated by data from Kolkata where again higher rates of *S. sonnei* (16.2%) were reported[20]. Overall, *S. flexneri* was most common (74.7%), with serotypes 2a (51%) and 3a (28.7%) being predominant among children less than 5 years[20]. In Global Enteric Multicenter Study, serogroups 2a (26.4%), 3a (12.1%), 6 (5.5%), 4a (5.5%), 7 (5.5%) and 1b (1.1%) were found in 91 *Shigella* isolates from Kolkata[21]. Another important feature was the emergence of untypeable shigellae. At our centre, over nine years, around 4 per cent isolates were untypeable[4]. Similar trends of emergence of untypaeble *Shigella* strains (13%) have been reported from Kolkata[20]. A peculiar observation from Kolkata was the absence of *S. dysenteriae* which was similar to our centre. In contrast, in Andaman islands in the Bay of Bengal over a period of seven years from 2006-2012, *S. dysenteriae* (12%) was recorded to be third most common, followed after *S. flexneri* (68%) and *S. sonnei* (20%)[22]. The heterogenous distribution of *Shigella* species and serogroups across the country implicates that multivalent or cross-protective *Shigella* vaccines are required to address the burden of shigellosis in India.

Host

Though humans and primates are the primary reservoir of *Shigella*[23], it has been isolated from

various sources *viz*. aquatic bodies (rivers, surface waters as well as coastal waters), free living amoebae, insects, birds and wild animals[24-31]. For a continuous transmission in humans, however, the bacterium must be passed from one person to another, as it does not survive in planktonic phase for long outside the body[23].

Routes of transmission/reservoirs and meteorological factors

The primary mode of transmission is by faeco-oral route and as low as 10-100 bacteria can cause infection[23]. Such a low infective dose enables *Shigella* to cause large outbreaks. The high incidence of *Shigella* in the developing world is generally attributed to lack of clean water, poor hygiene, malnutrition and close personal contact. Outbreaks have been associated with person-to-person transmission in crowded or unhygienic environments like prisons and asylums. Environmental factors such as rainfall and temperature have been shown to affect the transmission[32]. The expression of virulence genes is activated when bacteria are shifted from 30 to 37°C, in medium of moderate osmotic stress and *p*H 7.4[33]. *Shigella* infections can occur round the year, but peak prevalence in summer months has been reported[34]. In most communities the incidence is highest in hot and dry weather, possibly because the scarcity of water in such conditions limits hand-washing and other hygiene measures that reduce person-to-person transfer of the bacteria[23]. From Bay of Bengal islands, shigellosis is reported to occur mainly during rainy seasons, while low numbers of cases are recorded in winters[22].

Less commonly known route of transmission is through contaminated food and water or fomites. The ready-to-eat foods and beverages prepared by street-vendors can also be a source of shigellosis. In a study from Odisha in India, *panipuri* (a street food) samples examined for disease causing bacteria showed around 80 per cent to be coliform-positive with isolation of pathogenic bacteria like *Escherichia coli* (13.6%), *Klebsiella* sp. (10.6%), *Enterobacter* sp. (28.8%), *Bacillus* sp. (3%), *Enterococcus* sp. (6.1%), *Micrococcus tetragenus* (3%), *Salmonella* Paratyphi B (1.5%), *S. dysenteriae* (4.5%) and *Vibrio* sp. (6.1%)[35]. Similarly, food handlers in large food service establishments often carry pathogenic microorganisms and may be a source of infection to a huge number of people. At a tertiary care centre in north India, amongst asymptomatic food handlers, *Shigella* was the most common bacteria isolated[36].

In settings where disposal of human faeces is inadequate, flies, particularly *Musca domestica*, the common housefly, may serve as a vector for transmission of shigellosis[28]. When guts of houseflies collected from various public places including a garden, public park, garbage/dump area, hospital, restaurant/canteen, and human habitation in Pune, Maharashtra, India, were cultured, 102 bacterial strains were isolated, majority of which were known potential pathogens including *Shigella,* along with *Klebsiella*, *Aeromonas*, *Morganella*, *Providencia*, and *Staphylococcus*[28]. Also, animals in contact with human faeces may transiently harbour diarrhoeogenic bacteria as observed in Mumbai where *Shigella* as well as *Salmonella*, *Pseudomonas*, *Streptococcus*, *Proteus* and *Pasteurella* species were isolated from 200 pigs slaughtered at an organized slaughter house[30]. Shigellae have also been found in avian reservoirs in a zoological garden in Madagascar[29], and muscle tissue of several wild animal carcasses from wildlife species in Canada[31].

Several aquatic bodies have been found to show presence of shigellae and thus another potential source of infection may be aquatic food which may play a role in transmission of *Shigella* if such food is harvested from sewage-contaminated water[37]. The distribution of major groups of enteric bacteria was explored in sediments from coastal areas in south India from different depths (5, 15, 25 and 35 meter), and the flora was found to constitute *Vibrio parahaemolyticus*, *Shigella*, *Vibrio cholerae*, *Salmonella* and *E. coli* with higher bacterial populations in monsoon and pre-monsoon seasons[26]. Water samples of the river Narmada in Madhya Pradesh in India have been found to harbour *S. flexneri*, *S. sonnei* and *S. dysenteriae*. The virulence gene encoding the invasive plasmid antigen H (*ipaH*) was found in all the isolates, while the plasmid encoded invasion-associated genes (*ipaBCD*) were present only in *S. flexneri*, and the Shiga toxin (*stx*1) gene was found only in *S. dysenteriae*, thus demonstrating not only the existence of *Shigella* in the river but also the presence of an environmental reservoir of virulence genes[24]. Similarly, shigellae have been isolated from surface waters in Bangladesh[25]. *Shigella* species have also been found to grow symbiotically inside *Acanthamoeba castellanii* implicating that free-living amoebae may serve as a transmission reservoir for *Shigella* in water[27]. Such environmental gene pool may contribute to horizontal transfer of genes among strains and emergence of virulent strains leading to outbreaks[25], and may also

help the bacteria to survive in odd conditions and re-emerge in favourable conditions.

Risk groups

No individual is immune to shigellosis, but certain individuals are at increased risk. Worldwide, the incidence of shigellosis is highest among children less than five years of age, but it has been observed that during *S. dysenteriae* type 1 epidemics all age groups are affected[23]. The incidence of shigellosis has been reported to increase steadily after the age of 40, along with bacterial load as determined by semi-quantitative real-time PCR. This suggests that young children and old people shed the highest bacterial load and may be responsible for disproportional transmission of shigellosis[2]. In children who are malnourished, *Shigella* may cause a vicious cycle of further impaired nutrition, recurrent infection and growth retardation[23]. In the United States of America (USA) and Europe, children in day-care centers[38], migrant population, travellers to developing countries, persons in custodial institutions[39], prisoners and military personnel, and homosexual men are infected most often[23].

In HIV infected patients, intestinal parasitic pathogens are more common in antiretroviral therapy naive patients[40], however, the association of *Shigella* with HIV has not been properly studied in all patient groups. Association of shigellosis in men who have sex with men (MSM) has been implicated for a long time. In the 1970s and 1980s, shigellosis was identified as a potentially sexually transmitted disease among MSM[41], and increased incidence in men was attributed to the sexual practices of MSM[42]. A correlation between HIV infection and increased incidence of shigellosis from San Francisco area in 1996 suggested HIV as an important risk factor for shigellosis[43]. Another study from San Francisco in 1998-1999 linked the sexual practices of direct oral-anal contact in MSM to confer the highest risk and HIV infection likely contributing to increased host susceptibility[44]. Similar outbreaks of shigellosis among MSM have been reported from Canada, Australia, London, and Chicago[45-48].

The data for higher incidence of *Shigella* in association with HIV infection are not so convincing from India. The common enteric pathogens detected in 331 HIV infected patients presenting with diarrhoea in Pune were *Cystoisospora belli* (28%) and *Cryptosporidium parvum* (12%), whereas in HIV uninfected individuals *S. flexneri* (4.9%) was the second most common pathogen, only after *Entamoeba histolytica* (7.1%)[49]. In another study from New Delhi, *Shigella* was only found in 2 per cent cases of diarrhoea in HIV infected patients showing a similar incidence as in immunocompetent individuals[50]. In Senegal, *Shigella* was implicated as the most common cause (12.4%) of diarrhoea in immunocompetent adults but in immunocompromised individuals *Shigella* was fifth most common (7.6%)[51]. Shigellosis, presents as a more severe infection in HIV infected individuals, may cause bacteraemia and unusual features like keratitis and pneumonia, fail to respond to appropriate therapy thus requiring prolonged treatment, and may recur after completion of treatment[52-54].

Clinical features and complications

The clinical presentation ranges from watery, loose stools to severe symptoms such as fever, abdominal pain, tenesmus, and bloody diarrhoea. Severity of the disease varies by the infecting species - *S. dysenteriae* infections usually cause dysentery, which may also occur in infections caused by *S. flexneri*, whereas *S. boydii* and *S. sonnei* generally often self-limited watery diarrhoea[3]. Acute complications such as toxic megacolon, peritonitis, and septicaemia are mostly observed in severely malnourished children, though may occur in absence of early antibiotic treatment[23]. *Shigella* dysentery may also lead to dangerous complications such as persistent diarrhoea, severe anorexia, weight loss and malnutrition, dilation of the large intestine, seizures, kidney damage, and haemolytic-uremic syndrome[55]. Bacteraemia may be reported in infants and immunocompromised adults[56]. Pneumonia associated with *S. sonnei* has also been described in malnourished children, in HIV infected patients, and in patients with chronic diseases[54,57].

Mortality

The advent of oral rehydration therapy to correct dehydration due to diarrhoea has greatly decreased the number of deaths due to diarrhoea of any aetiology. Severe dehydration is uncommon in shigellosis, and with proper hydration, shigellosis is generally a self-limiting disease. However, in severe cases, without antimicrobial treatment the mortality of shigellosis is greatly increased[23]. The availability of age-specific estimates of case fatality due to shigellosis is limited. The death rate due to *Shigella* infection in developed countries is low (0.05 to 0.4%)[58]. The earlier data from developing countries over a period (1974-1988) have indicated that 13.9 per cent of infants and 9.4 per cent of 1-4 year-olds die annually due to shigellosis

as estimated in admitted patients at the International Centre for Diarrhoeal Disease Research (ICDDRB), Bangladesh[59]. However, there is a change in this situation in recent years where a decrease in mortality due to shigellosis has been observed as compared to the previous reports. In a multi-centric study from six Asian countries, no deaths were recorded in 845 patients[2]. The authors suggested adequate treatment to study participants and absence of *S. dysenteriae* type 1 as possible reasons for unexpectedly low morbidity and mortality in the survey[2].

Epidemiology

Shigellosis occurs worldwide, in endemic and epidemic forms. Majority of cases are children <5 yr of age. The annual number of shigellosis episodes throughout the world is estimated to be 164.7 million, with 69 per cent of all episodes and 61 per cent of all deaths attributable to shigellosis involving children <5 yr of age[58]. The multi-centric study from six Asian countries[2] estimated *Shigella* as the causative agent in 5 per cent of the diarrhoeal cases, indicating an overall incidence of treated shigellosis to be 2.1 episodes per 1,000 residents per year in all ages, with higher rates in children and people more than 40 yr of age[2]. Among children <5 yr old, the incidence was 13 new cases per 1,000 children per year[2]. From Bangladesh a trend of increasing number of patients ≥60 yr has been reported in an analysis of patients from 2001-2012 than children <5 yr and adults aged 5-59[59]. Reports of shigellosis from various parts of the country have shown an overall isolation rate of shigellae varying from 3-6 per cent of all stool samples with diarrhoea[4,20]. At our tertiary care centre in north India, shigellae have been detected from 3 per cent of diarrhoeal stool samples[4], whereas in Kolkata, Nair *et al*[20] isolated 6.1 per cent *Shigella* spp. from hospitalized diarrhoeal patients. In paediatric age group, again, higher isolation rates have been reported, 9.5 per cent in Bay of Bengal islands and 11.5 per cent in Kolkata[22,60].

Several epidemics have been reported from many Asian countries such as Bangladesh (1972-1978, 2003), Sri Lanka (1976), Maldives (1982), Nepal (1984-1985), Bhutan (1984-1985) and Myanmar (1984-1985)[61-64]. In India, epidemics have been reported from southern India, Vellore (1972-1973, 1997-2001)[65,66], eastern India (1984)[67,68], Andaman and Nicobar islands (1986)[69,70] and Chandigarh (2003) in northern India[14]. Epidemic dysentery caused by MDR *S. dysenteriae* serotype 1 has been a recurrent challenge.

Outbreaks: Shigellae have the ability to cause outbreaks involving a large number of people. In the 1984 outbreak in West Bengal and Tripura, 3,50,000 people were affected with 3500 deaths[68]. In the 2002 outbreak, which occurred in West Bengal and tea gardens of Siliguri, the overall attack rate was 25.6 per cent. The death rate among those admitted to hospital was 6 per cent and the overall case-fatality ratio was 0.9 per cent[71]. All these outbreaks were due to *S. dysenteriae* serotype 1. The re-emergence of *S. dysenteriae* type 1 with added resistance to ciprofloxacin which has epidemic potential, has also been reported from our centre[12]. Outbreaks caused by *S. flexneri* and *S. sonnei* have also been reported recently from various parts of the country like West Bengal (2007), Kerela (2009) and Maharashtra (2010)[72,73]. In 2007, in a municipality in West Bengal, *S. flexneri* serotypes 2a and 3a caused water borne outbreak affecting 461 persons. The sources associated with the illness were drinking, washing utensils and bathing in tap water contaminated with *Shigella*, and the outbreak subsided following repair of the pipeline[72]. Food-borne outbreaks of *S. sonnei* have also been reported. More than 300 people suffered in Kerala in south India in 2009 where local food made of rice, lentils, milk, and water was implicated as the source[73]. In Maharashtra in 2010, about 150 persons suffered from shigellosis after eating in a *madrasa* (a religious place)[73]. These reports support the extension of *S. sonnei* into India[73]. These outbreaks indicate that in India, food- and water-borne routes for transmission of shigellosis may not be so uncommon, and thus reinforces the need to provide adequate sanitation.

Traveller's diarrhoea: Shigellae are also important agents of diarrhoeal disease in travellers from developed world to other countries. As per an estimate, 15-20 million travellers to developing countries experience diarrhoea annually[74]. Of the 64,039 enteric infections reported to FoodNet with information about travel, 8270 (13%) were travel associated, and amongst the bacterial agents, *Shigella* (13%) was the third most common, after *Campylobacter* (42%), and non-typhoidal *Salmonella* (32%). The most common travel destinations were Mexico, India, Peru, Dominican Republic, and Jamaica[75].

Therapeutic challenge of multidrug resistant shigellae

The emergence of MDR *Shigella* has been reported from all over the world, including the USA, Iran, China,

Indonesia, Vietnam, Bangladesh and India[2,64,76,77]. The progressive development of antibiotic resistance in *Shigella* isolates, as in all other bacteria, is not a new phenomenon. Sulphonamides were the first drugs of choice when introduced in the early 1940s and all the *Shigella* strains were sensitive to this drug. In late 1940s sulphonamides became ineffective, and tetracycline followed by chloramphenicol were recommended for shigellosis. Soon, resistance to these two drugs was also observed and ampicillin and co-trimoxazole came to the rescue[78]. These were clinically highly effective. However, during the epidemic in eastern India in 1980s, the *S. dysenteriae* type 1 isolates were found to be resistant to most of the antibiotics except nalidixic acid which was found to be clinically effective[78,79]. Later, resistance to nalidixic acid appeared in *S. dysenteriae* type 1 isolates from an outbreak in Tripura in 1988[80]. In the late 1980s, fluoroquinolones (norfloxacin, ciprofloxacin and ofloxacin) were introduced in India and were found to be very effective for shigellosis, including MDR *S. dysenteriae* type 1[81].

In 1990, ciprofloxacin was recommended as the drug of choice for empiric treatment of shigellosis in view of the existing high level resistance to agents like chloramphenicol, ampicillin, co-trimoxazole and nalidixic acid[82]. Ciprofloxacin proved to be highly effective in the treatment of shigellosis, but possibly due to overuse and misuse of this agent promoted by the easy over-the-counter availability of antibiotics without prescription, resistance emerged against this agent also. Outbreak investigations in India (Chandigarh, Siliguri, Aizawl and Diamond Harbour, Kolkata) showed high level resistance to ciprofloxacin, norfloxacin and ofloxacin[71,77]. At our centre, an outbreak of ciprofloxacin-resistant *S. dysenteriae* serotype 1 occurred in 2003. There was no mortality associated with these cases and they were managed by cefotaxime and amikacin[14]. Thereafter, high levels of resistance to nalidixic acid (*S. dysenteriae* 81.8%, *S. flexneri* 74.1%) and ciprofloxacin (*S. dysenteriae* 54.5%, *S. flexneri* 45.6%) have consistently been noted in various serogroups of shigellae[16,83]. From north eastern region, an outbreak of bacillary dysentery caused by quinolone resistant *S. dysenteriae* type 1 was reported[84]. Another study from Kolkata reported MDR shigellae most of which were found resistant to fluoroquinolones *viz*. ciprofloxacin (90%), norfloxacin (83%), and ofloxacin (81%), however, majority were still susceptible to ceftriaxone (94%)[20]. At another hospital in Kolkata, majority of *Shigella* isolates (81%) were MDR and emergence of fluoroquinolone-resistant *S. dysenteriae* type 1 (100%) in 2002-2003 was followed by frequent isolation (>25%) of fluoroquinolone resistant *S. flexneri* 2a and 3a in 2004, which restricted use of fluoroquinolones for treatment of shigellosis[60]. Fluoroquinolones are thus no longer the preferred group of drugs for managing shigellosis in India.

World Health Organization (WHO) now recommends ceftriaxone, pivmecillinam and azithromycin as alternative drugs to fluroquinolone-resistant shigellae[85]. We found 15.1 per cent of *S. flexneri* isolates collected over a period of nine years (2000-2009) resistant to at least one of the third-generation cephalosporins (ceftriaxone/cefotaxime)[4]. The first isolate showing ceftriaxone resistance was obtained in 2001 and an increase in number of isolates resistant to third generation cephalosporins was observed 2005 onwards. This situation has now become a therapeutic challenge in this region. The minimum inhibitory concentration (MIC) values for *Shigella* isolates revealed a rise for ceftriaxone (MIC_{90}: 12 mg/l) and cefepime (MIC_{90}: 8 mg/l). MIC values for *S. dysenteriae* remained below 1 mg/l for ceftriaxone, however, for cefepime the MIC_{90} has raised to 4 mg/l. These infections caused by ceftriaxone resistant *S. flexneri* isolates were successfully treated by azithromycin at our center[4]. Emerging resistance to cephalosporins has also been reported from Puducherry and Manipal in south India[18,19]. Azithromycin has also now joined the exhaustive list of drugs to which *Shigella* has developed resistance[59].

MDR shigellae have also been reported from travellers who have visited India[86]. At Kansai Airport Quarantine Station in Japan, stool samples were collected from overseas travellers with a history of diarrhoea over the period 2001-2005, and 53-106 *Shigella* strains were isolated per year (average 82.4), about 80 per cent of which were *S. sonnei*, and the most frequent country of origin was India[86]. Such widespread resistance to almost all classes of drugs presently in use for shigellae narrows down the choice of effective antimicrobial agents for shigellosis and is a matter of concern.

Molecular epidemiology

There has been an upsurging interest in exploring the molecular epidemiology of genetically encoded virulence factors and antimicrobial resistance markers of *Shigella*. Such insights may be valuable in understanding the transmission patterns, severity

of clinical presentations and response of *Shigella* to antimicrobial drugs.

Molecular epidemiology of virulence genes: Several virulence factors have been reported in *Shigella*. Virulent genes of *Shigella* may be present in isolates from both symptomatic and asymptomatic people. In Kolkata, all 91 *Shigella* isolates from both cases and controls were positive for *ipaH* gene[87]. Other virulence genes such as virulence regulator (*VirF*), secreted autotransporter toxin (*sat*), *Shigella* enterotoxin 1 subunit A (*setA*), *Shigella* enterotoxin 1 subunit B (*setB*), *Shigella* enterotoxin 2 (*sen*) and epithelial cell penetration encoded by invasion associated locus (*ial*) were detected in *Shigella* isolates in 80.2, 49.4, 27.4, 27.4, 80.2 and 79.1 per cent of cases and in 64.7, 52.9, 17.6, 17.6, 64.7 and 64.7 per cent of controls, respectively. The *Shigella* pathogenicity island (SH-PAI) was detected exclusively in serotype 2a. Such asymptomatic carriers may play a crucial role in the transmission of shigellae in endemic communities[87].

Molecular epidemiology of antimicrobial resistance: Shigella has adapted well to many antimicrobial agents due to its ability to carry mobile genetic elements that may facilitate inter- and intra-species dissemination of antimicrobial resistance genes[88]. In Kolkata, integron carriage has been detected in high numbers in *Shigella* isolates from cases (76.9%) than from controls (3505%). Of these, atypical class 1 integron has been exclusively detected in *S. flexneri* from cases but not from the controls[87]. The main mechanism of quinolone resistance involves accumulation of sequential mutations in DNA gyrase and DNA topoisomerase IV. The plasmid-mediated quinolone resistance (PMQR) has been described due to mutations in the *aac(60)-Ib-cr* gene that encodes for a variant of aminoglycoside acetyltransferase, also known to reduce ciprofloxacin activity[89,90]. PMQR genes in shigellae have been reported from USA, Japan, China and India[87,91-93]. From north India, we screened *S. flexneri* (n=139) and *S. dysenteriae* serotype 1 (n=38) isolated over the period 2001-2011 for PMQR determinants, and found 6.2 per cent shigellae to harbour PMQR determinants, of which two were positive for the *qnrS1* gene while nine were positive for *aac(60)-Ib-cr* gene. A high MIC for ciprofloxacin (32 mg/l) was shown by four of the 11 PMQR-positive isolates, while majority of isolates with a ciprofloxacin MIC 1 mg/l were negative for PMQR determinants[83]. Although *qnrB* is the most common gene belonging to the *qnr* family, we detected *qnrS1*-positive *Shigella* from the Indian subcontinent in two strains from 2010, indicating a relatively new appearance of this PMQR determinant among *Shigella* in India[83]. After the description of *qnrS1*-harbouring *Shigella* in Japan, China and the USA, appearance of *qnrS1*-positive *Shigella* in India is a matter of concern. We also detected at least two mutations in the QRDRs of each of the 11 PMQR-positive strains by sequencing of *gyrA* and *parC* indicating that PMQR determinants may have provided selection advantage by contributing to reduced susceptibility to quinolones and thus facilitating the selection of *gyrA*/*parC* mutants. Besides, four of the PMQR-positive isolates were positive for $bla_{\text{CTX-M-15}}$, one of which was also positive for $bla_{\text{CMY-2}}$[85]. A close association of *aac(60)-Ib-cr* with $bla_{\text{CTX-M-15}}$ has been detected in *Shigella* and is of great concern as $bla_{\text{CTX-M-15}}$ has emerged worldwide in recent years. From Andaman and Nicobar Islands also, presence of *aac(60)-Ib-cr* and *qnrB* in *Shigella* has been reported, indicating a widespread presence of these resistance determinants in the country[22].

Resistance to cephalosporins is mediated by extended-spectrum beta lactamases (ESBLs) which hydrolyze a wide variety of penicillins and cephalosporins. The ESBL mediated resistance to third-generations cephalosporins arises from mutations which broadens the spectrum of native beta-lactamases like TEM-1, TEM-2, and SHV-1. Another resistance mechanism includes overproduction of chromosomal or plasmid-derived *AmpC* beta-lactamases[94]. We investigated the presence of antimicrobial resistance genes in 119 *S. flexneri* and 24 *S. dysenteriae* isolates over a period of nine years (2001-2009)[4], and found 20 *S. flexneri* isolates with high MICs for cephalosporins. Nine of the 20 isolates were found to be positive for ESBL and six for *AmpC* production by phenotypic tests, and among the resistance determinants bla_{TEM} closely resembling $bla_{\text{TEM-116}}$ was the most common ESBL gene present in all 20 isolates, followed by $bla_{\text{CTX-M-15}}$ in 10, bla_{OXA} in eight, and $bla_{\text{CMY-2}}$ in seven, while none was positive for bla_{SHV}[4]. In contrast, of the 88 isolates from Bay of Bengal islands, 2 per cent showed the presence of the bla_{SHV} gene, while all 15 third generation cephalosporins-resistant isolates showed the presence of the bla_{TEM}, bla_{OXA1}, and $bla_{\text{CTX-M3}}$ genes[22]. A high ESBL prevalence in members of the *Enterobacteriaceae* family has been reported in India[95] with $bla_{\text{CTX-M-15}}$ being the most common ESBL gene. The finding of a high prevalence of ESBL producing genes like $bla_{\text{CTX-M-15}}$ which spread by horizontal transfer and/or mobilization of genetic mobile elements by orofaecal route, has serious implications in terms of further

spread of resistance to third generation cephalosporins to other regions[96]. Overcrowding and poor sanitation, and the selective pressure due to overuse of antibiotics have been considered responsible for such widespread dispersal of $bla_{CTX\text{-}M\text{-}15}$[96].

From south India in Bay of Bengal islands, other resistance determinants were analyzed in 88 isolates and MDR was found to be associated with various drug-resistant genes[22]. Ampicillin resistance was largely associated with TEM β-lactamase genes (100% isolates), the most prevalent resistance gene in ampicillin-resistant *Enterobacteriaceae* while gentamicin resistance was associated with *aac2* gene in 22 per cent of the isolates. Plasmid mediated resistance was commonly found for tetracycline (*tetB* in 92% and *tetA* efflux genes in 90%), chloramphenicol (*catI* gene in 32%) and co-trimoxazole (*dfrA1* in 81% and *dfrA5* in 78%)[22]. Thus, there is a widespread emergence of MDR *Shigella* in the face of rampant injudicious antimicrobial use which reinforces the need for continuous surveillance of antimicrobial resistance determinants across the country to know the molecular epidemiology of resistance, which is further essential for implementing timely intervention steps to control the disease as well as spread of these resistance genes to other parts of the world.

Prevention and control

The most effective measure to decrease transmission of shigellosis is proper washing of hands, especially after defaecation. Community health education must emphasize upon good personal hygiene, adequate disposal of faeces, as well as the imminent threat of MDR pathogens. The widespread practice of misuse of antibiotics in viral diarrhoea should be discouraged by means of education as well as legislation. Better awareness about general measures such as washing, peeling and cooking of all fruits and vegetables, avoidance of food preparation by personnel who change diapers in daycare centres, proper handling and refrigeration of food, encouraging prolonged breastfeeding in infants, and appropriate case reporting to health authorities may be helpful to prevent further transmission[85]. Though public health measures to reduce exposure and transmission are highly effective, the establishment of such infrastructure in developing countriesis resource intensive and thus remains challenging.

Vaccines: There is a strong need for an effective, safe and cheap vaccine against shigellosis[2]. The high disease burden of shigellosis in developing countries, children <5 yr of age as the main victims, difficulty in achieving adequate sanitation and personal hygiene in these regions and scarce therapeutic alternatives for emerging MDR *Shigella* point towards vaccination as a hope for effective and sustainable strategy against shigellosis. Shigellosis is targeted by WHO as one of those enteric infections for which new vaccines are most needed, the target populations being travellers from developed countries and military service personnel, as well as children living in endemic areas[2,58].

Although the need for a *Shigella* vaccine is urgent, not much progress has been done due to the antigenic complexity, lack of inter-species cross-protective epitopes, and gaps in understanding of the protective immune response. Several different types of vaccines against *Shigella* have been experimentally tested in animal models and in volunteer trials[12,97]. Various live attenuated vaccines such as CVD103, CVD104, CVD107, CVD108, SC602 and WRSS1 have been developed in the past[12], however, most were serotype-specific with no cross-protectivity. These vaccines progressed into phase 1/2 trials but none could go beyond[12]. (Ipa, B, C) subunit vaccine approach has been used for Invaplex (*Shigella* invasion complex) containing invasion plasmid antigens B and C, and lipopolysaccharide (LPS), which was found to induce protective immunity in experimental animals[98]. Similarly, outer membrane proteins are being developed as attractive vaccine options[99]. However, apart from a live, non-invasive *S. flexneri* 2a-*S. sonnei* bivalent vaccine used in China, there are currently no licensed vaccines available[100]. The current vaccine candidates are either not sufficiently attenuated or not properly immunogenic[101]. Thus, the goal for an effective, safe and successful multivalent vaccine targeting prevalent species and serotypes is yet to be achieved[98,102]. Livio *et al*[21] have shown that a quadrivalent vaccine with O antigens from *S. sonnei*, *S. flexneri* 2a, *S. flexneri* 3a, and *S. flexneri* 6 may be effective against these most common serotypes.

Conclusion

Shigellosis is one of the major causes of diarrhoea in India. *S. flexneri* is the most common species present in the country, *S. sonnei* and non-agglutinable shigellae are steadily surfacing, while *S. dysenteriae* has temporarily disappeared from northern and eastern regions. Though shigellosis appears to be endemic and has been reported to cause many outbreaks, the accurate estimates of morbidity and mortality are lacking. The

limited information available indicates *Shigella* to be an important food-borne pathogen in India. There is a nationwide presence of MDR shigellae developing rapid resistance to most antibiotics available. Thus, judicious use of antibiotics for *Shigella* is amongst the most essential measures to combat shigellosis. This calls for a continuous and strong surveillance of antibiotic resistance across the country for periodic updation of the local antibiograms. The prevention of shigellosis is desirable as it will substantially reduce the morbidity associated with diarrhoea in the country. Public health measure like provision of safe water and adequate sanitation are of immense importance to reduce the burden of shigellosis, however, the provision of resources to develop such infrastructure to the huge population of a country like India is a complex issue and will take time to resolve, hence focusing the scientific thrust towards development of a safe and affordable multivalent vaccine may be the need of the hour.

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