Abstract

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. Among others, Shigellosis represents a major burden of disease, especially in developing countries, and is estimated to affect more than 160 million human beings are infected by the microorganism annually and that approximately 1.1 million die.

Materials & Methods: The study was undertaken at the Department of Microbiology in Maulana Azad Medical College & Lok Nayak Jai Prakash Hospital, tertiary care, New Delhi India, from January 2012 to December 2016. The samples were processed according to standard bacteriological procedure. Resistance patterns of the shigella isolates to various antibiotics were determined by the agar diffusion technique.

Results: A total of 9577 stool specimens were collected, of diarrhea /dysentery patients during the study period (January 2012 to December 2016). A total of 100 strains of shigella species were isolated thus showing a prevalence of 1.04%. The commonest species isolated was S. flexneri (55%), followed by S. boydii (19%), S. sonnei (15%) and S. dysentery (11%). All the isolates were sensitive to erythromycin, trimethoprim-sulfamethoxazole and aztreonam and showed variable resistance against the remaining antibiotics.

Conclusion: Limited laboratory diagnosis in developing countries imposes clinicians to syndromic diagnosis and empirical prescription of broad spectrum antibiotics that led drug resistant bacterial strains to emerge. More emphasis should be given towards supply of safe water and health education for the community.

Keywords: Antimicrobial susceptibility pattern, Diarrhea, Trend of 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. Although shigellosis is associated with a few medical complications only, adequate control of this disease may reduce the overall diarrhea burden globally. The diagnosis of shigellosis is made by culture isolation of shigella from feces 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. Among...
others, Shigellosis represents a major burden of disease, especially in developing countries, and is estimated to affect more than 160 million human beings are infected by the microorganism annually and that approximately 1.1 million die.\(^3\) Disease may be caused by any of the 4 shigella species: \textit{S. dysenteriae}, \textit{S. flexneri}, \textit{S. boydii}, and \textit{S. sonnei}. Among the \textit{shigella}, \textit{S. flexneri} predominates in developing countries and \textit{S. sonnei} in industrialized countries.\(^5\) However, empiric antimicrobial therapy requires knowledge of the local antibiogram of circulating \textit{shigella} strains. Of growing concern is multidrug resistance, and in particular the increasing rate of resistance to ciprofloxacin reported for \textit{shigella} isolates from Asian and African regions.\(^4\) Furthermore, resistance to recommended second-line antimicrobial drugs, such as the third-generation cephalosporin ceftriaxone, cefotaxime and the macrolide (azithromycin), is emerging of multidrug resistant (MDR) \textit{shigella}, notably the increasing resistance to third generation cephalosporin’s and fluoroquinolones, and most recently azithromycin.\(^2,5,7\) In this review, we will focus upon shigellosis in Indian perspective, mainly addressing the epidemiological parameters, disease burden, and the therapeutic challenges of emerging Multi-drug resistant (MDR) \textit{shigella}.

Materials and Methods

Patients and Sample Collection

The study was undertaken at the Department of Microbiology in Maulana Azad Medical College & Lok Nayak Jai Prakash Narayan Hospital, tertiary care, New Delhi India, from January 2012 to December 2016. The Ethics Committee of the institute approved the study. A total of 100 suspected cases were included in the present study based on the inclusion criteria: Any age, either sex, of diarrhea (passing stools at least 3 times in 24 hours) or dysentery (bloody stools or mucoid stools), and stool culture positive for \textit{shigella} species. Informed and written consents were obtained from the parents or guardian. The sample was collected in a clean, dry and open-mouth disposable container. All the samples were cultured within 2 hour of collection and analyzed according to standard methods.\(^8\) Though most of the patients had suffered from dysentery, some patients had only mild diarrhea and never developed dysenteric symptoms. Dysentery was characterized by frequent passage (usually 10 to 13 times/day) of small volume stools consisting of blood, mucus, and pus; often accompanied by abdominal cramps and tenesmus. Diarrhea was defined as the passage of 3 or more liquid stools without blood and mucus in a 24 h period.

Bacteriological Processing of Stool Specimens

The samples were primarily cultured on deoxycholate citrate agar (DCA) and MacConkey agar media (HiMedia Laboratories Pvt. Ltd.). All plates were incubated aerobically at 37°C overnight. The non-lactose-fermenting (NLF) colonies from both DCA and MacConkey agar were identified on urea hydrolysis, triple sugar iron (TSI) medium, hanging drop method for motility and Simmons’s citrate test.\(^9\) Serogroups of \textit{Shigella} was identified by slide agglutination test with specific antiserum (DIFCO Laboratories, Detroit, Michigan (USA). Serotyping was done by latex agglutination for detecting serogroups included serogroup A: \textit{Shigella dysenteriae}, serogroup B: \textit{Shigella flexneri}, serogroup C: \textit{Shigella boydii}, serogroup D: \textit{Shigella sonnei}. All procedure was done according to manufactures instruction.\(^10\)

Antimicrobial Susceptibility Testing

Resistance patterns of the \textit{shigella} isolates to various antibiotics were determined by the agar diffusion technique.\(^11,12\) Every inoculum was prepared by inoculating 5 ml of Mueller-Hinton broth with five colonies of an 18-24 hours old pure \textit{shigella} culture followed by incubation in ambient air and at 37°C for 16 h. The resulting turbid culture was standardized to a turbidity of 0.5 McFarland using 0.85 per cent sodium chlorides as a diluent. A sterile cotton swab was dipped into the standardized suspension, drained, and used for inoculating 25 ml of Mueller-Hinton agar (MHA) in a 90 mm plate. The inoculating plates were air dried and antibiotic disks included ampicillin (10 μg), tetracycline (30 μg), cotrimoxazole i.e. trimethoprim/ sulphamethoxazole (1.25/23.75 μg), cefotaxime (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), nalidixic acid (30 μg), chloramphenicol (30 μg) amikacin (10 μg) and gentamicin (10 μg) Hi-Media (Mumbai, India) were mounted on them.\(^12,13\) The plates were inverted and incubated in ambient air at 37°C for 18 hours. The inoculums for the susceptibility testing and the interpretation were done as per CLSI 2017 (Clinical Laboratory Standards Institute) guidelines.

Quality Control and Data Analysis

A standard bacteriological procedure was followed to keep the quality of all laboratory tests. American Type Culture Collection (ATCC) strains (\textit{Shigella sonnei} ATCC 25331 and \textit{Escherichia coli} ATCC 25922) were used as control strains for the culture and sensitivity testing. data was captured in WHONET and was analyzed. All The significance of differences between percentages of antimicrobial resistance of \textit{Shigella} species was determined by the chi-square test or the Fisher’s exact test. P-value <0.05 was considered statistically significant.

Results

A total of 9577 stool specimens were collected, of diarrhea/dysentery patients during the study period (January 2012 to December 2016). A total of 100 strains of \textit{shigella} species were isolated thus showing a prevalence of 1.04% (n=9577) among the all population irrespective of age/sex of hospital
attending patients. The commonest species isolated was *S. flexneri* (55%), followed by *S. boydii* (19%), *S. sonnei* (15%) and *S. dysentery* (11%). A significant outbreak of shigellosis 37 cases (37%) occurred in 2012 followed by sporadic cases in 2015 (8 cases) throughout 2016. (Figure 1) Our study shows that *S. flexneri* 2a (35%), 3a, 6 and 2b was the most common serotype followed by *S. dysenteriae*. (8%) A change in resistance patterned was observed in antibiotics.

![Figure 1. Prevalence of *shigella* species from January 2012 to December 2016 (n=100)](image)

**Table 1. Distribution of *shigella* species according to age groups**

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th><em>S. flexneri</em> n (%)</th>
<th><em>S. boydii</em> n (%)</th>
<th><em>S. dysenteriae</em> n (%)</th>
<th><em>S. sonnei</em> n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6 months</td>
<td>4 (7.2)</td>
<td>2 (10.5)</td>
<td>3 (20)</td>
<td>2 (18.1)</td>
<td>11 (11)</td>
</tr>
<tr>
<td>6 months – 2 years</td>
<td>5 (9.0)</td>
<td>3 (15.7)</td>
<td>2 (13.4)</td>
<td>3 (27.2)</td>
<td>13 (13)</td>
</tr>
<tr>
<td>2-5 years</td>
<td>12 (21.8)</td>
<td>4 (21.05)</td>
<td>2 (13.4)</td>
<td>2 (18.1)</td>
<td>20 (20)</td>
</tr>
<tr>
<td>5-10 years</td>
<td>8 (14.5)</td>
<td>2 (10.5)</td>
<td>1 (6.7)</td>
<td>1 (9.09)</td>
<td>12 (12)</td>
</tr>
<tr>
<td>11-20 years</td>
<td>7 (12.7)</td>
<td>3 (15.7)</td>
<td>2 (13.4)</td>
<td>2 (18.1)</td>
<td>14 (14)</td>
</tr>
<tr>
<td>21-30 years</td>
<td>11 (20)</td>
<td>2 (10.5)</td>
<td>2 (13.4)</td>
<td>0 (0)</td>
<td>15 (15)</td>
</tr>
<tr>
<td>31-40 years</td>
<td>6 (10.9)</td>
<td>2 (10.5)</td>
<td>1 (6.7)</td>
<td>1 (9.09)</td>
<td>10 (10)</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>2 (3.6)</td>
<td>3 (15.7)</td>
<td>2 (13.4)</td>
<td>0 (0)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Total</td>
<td>55 (55%)</td>
<td>19 (19%)</td>
<td>15 (15%)</td>
<td>11 (11%)</td>
<td>100 (100%)</td>
</tr>
</tbody>
</table>

**Table 2. Antibiogram of *shigella* strains from January 2012 to December 2016 (n=100)**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Breakpoint</th>
<th>Number</th>
<th>%R</th>
<th>%I</th>
<th>%S</th>
<th>%R, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (10 µg)</td>
<td>18-21</td>
<td>91</td>
<td>100</td>
<td>13.3</td>
<td>12.2</td>
<td>5.5-100</td>
</tr>
<tr>
<td>Amoxicillin (10 µg)</td>
<td>14-17</td>
<td>20</td>
<td>74.4</td>
<td>0</td>
<td>30</td>
<td>45.7-87.2</td>
</tr>
<tr>
<td>Cefazidime (30 µg)</td>
<td>18-20</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>5.5-100</td>
</tr>
<tr>
<td>Cefotaxime (30 µg)</td>
<td>23-25</td>
<td>100</td>
<td>40</td>
<td>7</td>
<td>53</td>
<td>30.5-50.3</td>
</tr>
<tr>
<td>Aztreonam (30 µg)</td>
<td>18-20</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0.0-94.5</td>
</tr>
<tr>
<td>Imipenem (10 µg)</td>
<td>20-22</td>
<td>2</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>2.7-97.3</td>
</tr>
<tr>
<td>Amikacin (30 µg)</td>
<td>15-16</td>
<td>99</td>
<td>4</td>
<td>10.1</td>
<td>85.9</td>
<td>1.3-10.6</td>
</tr>
<tr>
<td>Gentamicin (30 µg)</td>
<td>13-14</td>
<td>100</td>
<td>23</td>
<td>10</td>
<td>67</td>
<td>15.4-32.7</td>
</tr>
<tr>
<td>Tobramycin (30 µg)</td>
<td>13-14</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>5.5-100</td>
</tr>
<tr>
<td>Ciprofloxacin (5 µg)</td>
<td>16-20</td>
<td>100</td>
<td>63</td>
<td>16</td>
<td>21</td>
<td>52.7-72.3</td>
</tr>
<tr>
<td>Norfloxacin acid (30 µg)</td>
<td>17-20</td>
<td>58</td>
<td>58</td>
<td>12</td>
<td>30</td>
<td>28.3-45.7</td>
</tr>
<tr>
<td>Trimethoprim-Sulfamethoxazole (1.25/23.75 µg)</td>
<td>11-15</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0.0-94.5</td>
</tr>
<tr>
<td>Erythromycin (30 µg)</td>
<td>14-22</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0.0-94.5</td>
</tr>
<tr>
<td>Tetracycline (10 µg)</td>
<td>20-24</td>
<td>12</td>
<td>68</td>
<td>18</td>
<td>24</td>
<td>0.12-84.</td>
</tr>
</tbody>
</table>
Male’s children outnumbered the females by M: F=1.2:1. The predominant age group of patients who were positive for Shigella belonged between 0 year to 5 years (54%) and the least frequent affected age group were more than 40-year (7%) Table 1.

Shigellosis were more common in summer season (68%) followed by winters and rainy weather.

**Antibiotic Susceptibility Pattern of the Strains Isolated**

All the isolates were sensitive to erythromycin, trimethoprim-sulfamethoxazole and aztreonam and showed variable resistance against the remaining antibiotics. Kirby-Bauer disc diffusion method demonstrated none of isolates were susceptible to ampicillin, ceftazidime and Tobramycin. The marked drug resistance was observed in shigella isolates to amoxicillin (74.4%), tetracycline (68%), ciprofloxacin (63%), nalidixic acid (58%), imipenem (50%) and cefotaxime (40%). Amikacin (4%) and gentamicin (23%) had the least resistance (Table 2). Overall, Shigella strains showed a statistically significant increase in resistance to amoxicillin, tetracycline, nalidixic acid and TMP-SMX (p<0.05) during our study.

Antimicrobial resistance varied by species (Table 3); most of the S. flexneri isolates were resistant to amikacin (94%), ampicillin (89%), and gentamicin (69%), whereas resistance to these antibiotics was much less common among S. sonnei isolates (84%, 91%, and 84%, respectively). In other words, S. flexneri was more resistant to antibiotics than S. sonnei. However, this difference was not significant (P value >0.05). Ceftazidime were 100% resistant to all shigella species. An isolate was defined as being multidrug resistant if it is resistant to three or more of the antimicrobial agents tested of the Shigella isolates, 17% were resistant to two or more antibiotic. Further analysis revealed that nearly 35% of isolates were resistance to two or more drugs (multidrug resistance).

**Discussion**

Shigellosis occurs both in epidemic and endemic forms in children and is a major public health problem in developing countries. Shigellosis still accounts for a significant proportion of morbidity and mortality, especially in developing countries. Over the past decades, Shigella spp. have become progressively more resistant to most of the widely used and inexpensive antimicrobials. Changes in the incidence of Shigella sero-groups also makes it difficult to formulate a drug of choice for shigellosis. The frequency of occurrence of Shigella species differs by country and in different populations within a country. The isolation rate of Shigella varies from 2-6 per cent in different studies across India. In present study, the prevalence of Shigella spp. was 1.04%, which was lower compared with reports of other similar studies in southern and northern parts of India. The distribution of Shigella species varies geographically both within countries and between countries. Shigellosis is predominantly caused by S. sonnei in industrialized countries, whereas S. flexneri prevails in the developing world. In this study, S. flexneri (55%, n = 100) was the most common, followed by S. byodii (19%, n=100), S. dysenteriae (15%, n = 100), and S. sonnei (11%, n = 100). S. flexneri was found to be the most prevalent serogroup (87.3%) in our study similar with other studies were conducted from various part of India. This may suggest the possible replacement of S. flexneri by S. sonnei in some part of India as the standard of living improved, as inferred from observations obtained from developing countries.
In areas where shigellosis is endemic, the highest rates of the infection occur within 5 years of life.\(^3,18\) We noted similar data: most of the cases of shigellosis were identified in children aged from 6 to 24 months. The predominant age group of patients who were positive for *Shigella* belonged between 0 year to 5 years (54%) and the least frequent affected age group were more than 40 year (7%). In areas where shigellosis is endemic, the highest rates of the infection occur in the second year of life.\(^3,18\) We noted similar data: most of the cases of shigellosis were identified in children aged from birth up to 5 years. The existence of several serotypes of the microorganism allows one to assume that, in the endemic regions, several episodes of the disease occur in childhood. After birth, although babies immunologically naive, but they would be come into contact with the microorganism more frequently. Consequently, they are more susceptible to the infection and develop the disease and progressive protection against the types of the microorganism circulating in that region. Thus, it is possible to explain the high frequency of shigellosis up to 5 years of ages.

Our study noticed that shigellosis was more common in the summer season it could be due to owing to the greater recreational use of water and the precarious hygiene habits which facilitate the transmission of diarrhoegenic bacteria. This finding correlates with the study conducted by Naumova et al.\(^23\)

Changing patterns of antimicrobial susceptibilities among *Shigella* isolates pose major difficulties in selecting an appropriate drug for the treatment of shigellosis.\(^31\) Currently in India, third-generation cephalosporin’s are used as an alternative in patients who do not respond to fluoroquinolone treatment.\(^24\) The acquisition of resistance by enteric pathogens to an increasing number of antibacterial drugs is becoming a grave concern, particularly in developing countries where shigellosis is common. The options for effective and inexpensive antibacterial therapy for shigellosis are shrinking. A network of laboratories for real-time monitoring of antibiotic resistance among enteric pathogens and timely dissemination of such information to the clinicians for modification of treatment strategy are greatly needed.

**Conclusion**

We suggest that periodic monitoring of the susceptibility patterns of these *Shigella* stains is important and should be undertaken in other centers so that larger multi-centric data are available to study the emergence of drug resistance to commonly used drugs and for choosing appropriate antimicrobial therapy for these infections. The overuse and misuse of antibiotics in the treatment of diarrhea could lead to an increase of antibiotic resistance. Limited laboratory diagnosis in developing countries imposes clinicians to syndromic diagnosis and empirical prescription of broad spectrum antibiotics that led drug resistant bacterial strains to emerge. More emphasis should be given towards supply of safe water and health education for the community. Accurate diagnosis during management of infection caused by *Shigella* should be employed than empirical treatment of patients. Periodic epidemiological surveillance is of great importance to control the diseases and MDR of *Shigella* spp.

**Conflict of Interest:** None

**References**

12. National Committee of Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing: ninth international supplement. Wayne,

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