Epidemiology of Shigella species isolated from diarrheal children and drawing their antibiotic resistance pattern

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ABSTRACT

Background: Shigellosis accounts for majority of cases of bacterial diarrhea in infants in developing countries. The present study was conducted to determine the distribution and pattern of antimicrobial resistance of Shigella species among children 0-14 years of age with acute diarrhea in Hamadan.

Materials and methods: The study included all acute diarrhea patients who admitted in the pediatric department from January 2001 to December 2004. Antibiogram test was performed by gel-diffusion method and ten commonly used antibiotics were applied.

Results: Of 1686 stool samples, 166 (9.8%) were positive for Shigella species. Shigella flexneri was the predominant serogroup (40.3%) followed by S. dysentriae (33.7%), S. boydii (15.1%) and S. sonnei (10.8%). Of Shigella isolates, 91% were resistant to one or more antimicrobial agent(s), and 88% were multi-drug resistant. Most strains were resistant to chloramphenicol (90%), ampicillin (89%), co-trimoxazole (84%), tetracycline (83%) and nalidixic acid (51%). Resistance to amoxicillin-clavulanic acid (co-amoxiclav), ceftriaxone, amikacin, nitrofurantoin and ciprofloxacin was observed in 34.9%, 23.4%, 6.6%, 3.6% and 1.8% of the isolates, respectively. Emerging resistance against nalidixic acid (42.3%) was observed.

Conclusion: Our experiences suggest that Shigella species could be an important etiological agent of diarrhea in this area, while the drugs of choice for the treatment of Shigella infection should be ciprofloxacin and nitrofurantoin. Amikacin was the third drug of choice.

Keywords: Diarrhea, Shigella, Antibiotic, Resistance, Children.

INTRODUCTION

Diarrheal diseases remain a major cause of morbidity and mortality in all age groups, especially in developing countries including Iran (1,2). In our country, diarrhea is estimated for the third leading cause of overall morbidity and the leading cause of infant mortality (3).

Shigellosis occurs both in epidemic and endemic forms in children and remains a major public health problem in developing countries (4,5). In a study conducted from 1997 to 1999, Shigella (S.) flexneri was found to be the most frequently isolated organism from diarrheal patients in a community setting in Jakarta (6).
Similarly, S. flexneri was the most common organism isolated in four low socio-economic areas of Karachi between January 2002 and March 2003 (7). The attack rate of shigellosis in India is 1-15% and bacillary dysentery is responsible for approximately 10% of deaths in children (2,8,9).

In another study from Bangladesh (10), Shigella species were the most common isolated species from patients with acute diarrhea and S. flexneri (54 %) was the most frequently isolated one, followed by S. dysenteriae, S. boydii and S. sonnei. An investigation from Bahia, Brazil was also revealed that the shigellosis was the predominant cause of acute diarrhea, while S. sonnei (80.1%) was the most common isolated organism followed by S. flexneri (19.9%) (5). Shigellosis is also an important cause of infectious diarrhea in Iran (3), mostly community-acquired, caused mainly by S. flexneri and S. dysenteriae.

Over the past decades, Shigella species have become progressively resistant to most of the widely used and inexpensive antibiotics (11-14). Resistance has emerged even to newer, more potent antimicrobial agents. Moreover, a change in the incidence of Shigella subgroups from time to time makes it difficult to formulate a drug of choice for Shigellosis (2,15,16). Prior to this study, the antibiotic resistance pattern of Shigella isolates has not been previously determined in this region. Therefore, this study was carried out to identify and to establish the antimicrobial resistance pattern of the most important Shigella serotypes involved in the epidemiology of acute diarrhea in children who admitted in the pediatric department of hospitals in Hamadan.

PATIENTS and METHODS

A prospective study was conducted on children with acute diarrhea and dysentery between 0 and 14 years of age from January 2001 to December 2004 who were admitted in the pediatric departments of two hospitals (Ghaem and Ekbatan) in Hamadan. A total of 1686 stool samples were examined for Shigella species, then isolates were serotyped and their antibiotics susceptibilities were determined. Only one Shigella isolate per patient per diarrheal episode was included in the analysis. No outbreak has been detected during the study period.

Fecal samples were collected in Cary-Blair transport medium and transferred immediately to the laboratories of hospitals. Cultivation and isolation of organisms were performed according to the methods outlined in the Bailey & Scott’s Diagnostic Microbiology (17). The samples were cultured into GN broth and plated onto Salmonella-Shigella (SS) agar and MacConkey agar (Merck, Germany). Plates were incubated at 37°C for selective isolation of Shigellae and were examined after 18-24h of incubation. Suspected colonies were inoculated into Triple sugar iron agar (Oxoid), Mannitol motility medium (Hi-media, India), Urea medium (Hi-media), and peptone water (1% Bactopepton, Difco; pH 7.2) for biochemical identification of Shigellae.

Shigella serotypes were identified using standard commercially available polyvalent and monovalent antisera (Wellcome Research Laboratories, Beckenham, UK). Single colonies of isolates were tested serologically by slide and tube agglutination with specific antisera against Shigella sonnei, Shigella flexneri, Shigella boydii and Shigella dysenteriae, according to the standard protocols (17).

In order to draw the susceptibility patterns of isolates, they were tested by disk diffusion method using guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS) (18). A total of 10 selected antibiotic disks (Mast Group LTD, UK) including chloramphenicol (CHL, 30µg), co-amoxiclav (AMC, 20µg), amikacin (AMK, 30µg), ampicillin (AMP10µg), ceftriaxone (CRO, 30µg), ciprofloxacin (CIP, 5µg), co-trimoxazole (TMP-SXZ, 25µg), nitrofurantoin (NIT, 300µg), nalidixic
acid (NAL, 30µg) and tetracycline (TET, 30µg) were applied during the test. The organisms used for quality control were Escherichia coli (ATCC 25922; American Type Culture Collection) and Staphylococcus aureus (ATCC 25923).

The initial data including patient's age and sex as well as types of microorganisms isolated from stool cultures and their antimicrobial resistance patterns were recorded in a special questionnaire. Finally, data analysis was achieved by SPSS software package (version 13.0, SPSS Inc., USA) and discrete variables were compared by the χ² test.

RESULTS

During the study period, of 1686 stool samples, 166 (9.8%) were positive for Shigella species. S. flexneri (40.3%) was the predominant serogroup followed by S. dysenteriae (33.7%), S. boydii (15.1%) and S. sonnei (10.8%). A number of serotypes were isolated in each serogroup, 7 serotypes in S. flexneri, 5 in S. dysenteriae, 4 in S. boydii, and Phase 1 & 2 in S. sonnei. The most common S. flexneri serotypes were 2a (31.3%), 1b (29.8%), and 2b (10.4%). The least common S. flexneri serotype was 1a (4.4%). The most common S. dysenteriae serotypes were type 1 (41.1%), type 2 (21.4%), and type 4 (16.1%), while type 7 (5.3%) was the least common. Table 1 represents the serotype distribution of shigella species (each isolate representing a case). Most of shigella species were isolated from patients during the first two years of study, 2001 and 2002 (25.9% and 30.7%), respectively.

Of 166 patients, 89 (53.6%) were male, and 15 (9.1%) were younger than two years of age, while 41 (24.7%), 54 (35.5%) and 51 (30.7%) aged 3-6, 7-10, and 11-14 years, respectively. Table 2 presents the distribution frequencies of the age groups of patients with Shigella. As shown in this table, S. flexneri and S. dysenteriae were more frequently observed in 7-10 and 11-14 year-old group, respectively.

Of the Shigella isolates, 82.5% were resistant to one or more of all tested antibiotics in this study (S. dysenteriae 100%, S. flexneri 90%, S. sonnei 80% and S. boydii 60%). Most strains were resistant to chloramphenicol (90%), ampicillin (89%), co-trimoxazole (84%), tetracycline (83%) and nalidixic acid (51%). Resistance to co-amoxiclav,
ceftriaxone, amikacin, nitrofurantoin and ciprofloxacin was observed in 34.9%, 23.4%, 6.6%, 3.6% and 1.8% of the isolates, respectively. Emerging resistance against nalidixic acid (42.3%) was observed. The antibiotics resistance distribution of Shigella isolates is shown in Table 3. Shigella flexneri, S. dysenteriae and S. sonnei shared very similar susceptibility profile for most of the tested antibiotics. The most frequent patterns of resistance were exhibited towards chloramphenicol, ampicillin, co-trimoxazole, tetracycline and co-amoxiclav (Table 3). S. boydii showed slightly different susceptibility profile. Resistance to nalidixic acid and ceftriaxone were appeared to be different in four types of Shigella isolates. S. dysenteriae isolates were more resistant than isolates of S. flexneri, S. sonnei and S. boydii to nalidixic acid (85.5% versus 44.7%, 38.8% and 0.0%, respectively, p<0.001). S. dysenteriae isolates also were more resistant than other three types of Shigella isolates to ceftriaxone (41.1% versus 14.9%, 0.0% and 12.0%, p<0.001).

Seventy eight percent of the Shigella isolates were multi-antibiotics resistant (resistance to at least two antibiotics). Different resistance patterns were defined in the four categories of the Shigella isolates. S. dysenteriae showed the highest multi-antibiotics resistant, while S. boydii showed the lowest. Twelve resistance patterns were observed in S. dysenteriae, while 10 R-patterns in S. flexneri, 9 in S. sonnei and 7 in S. boydii. The most prevalent multi-antibiotic resistance pattern was CHL*, AMP*, TMP-SXT*, TET in all types of Shigella isolates, namely, 37.6%, 32.6%, 28.2% and 19.4% for S. dysenteriae, S. flexneri, S. sonnei and S. boydii, respectively. The CHL*, AMP*, TMP-SXT*, TET*, NAL*, AMC pattern in S. flexneri strains and the CHL*, AMP*, TMP-SXT*, TET*, NAL*, CRO pattern in S. dysenteriae strains were the second most prevalent multi-antibiotic resistance patterns with a prevalence of 18.4 and 16.2% in each category, respectively. Three S. dysenteriae strains were resistant to all tested antibiotics, however, 2 S. boydii strains were susceptible to all tested antibiotics.

**Table 3. Distribution frequencies of the antibiotic resistance of 166 isolated Shigella species**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>S. flexneri</th>
<th>S. dysenteriae</th>
<th>S. boydii</th>
<th>S. sonnei</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol(30)</td>
<td>61(91.1)</td>
<td>52(92.8)</td>
<td>21(84.0)</td>
<td>17(94.4)</td>
<td>151(90.9)</td>
</tr>
<tr>
<td>Ampicillin (10)*</td>
<td>63(94.0)</td>
<td>54(96.4)</td>
<td>17(68.0)</td>
<td>15(83.3)</td>
<td>149(89.7)</td>
</tr>
<tr>
<td>Co-trimoxazole (25)</td>
<td>59(88.1)</td>
<td>52(92.8)</td>
<td>15(60.0)</td>
<td>14(77.7)</td>
<td>140(84.3)</td>
</tr>
<tr>
<td>Tetracycline (30)</td>
<td>61(91.1)</td>
<td>50(89.2)</td>
<td>13(52.0)</td>
<td>15(83.3)</td>
<td>139(83.7)</td>
</tr>
<tr>
<td>Nalidixic acid (30)</td>
<td>3(44.7)</td>
<td>48(85.7)</td>
<td>0</td>
<td>7(38.8)</td>
<td>85(51.2)</td>
</tr>
<tr>
<td>Co-amoxiclav (20)</td>
<td>20(29.8)</td>
<td>21(37.5)</td>
<td>10(40.0)</td>
<td>7(38.8)</td>
<td>85(51.2)</td>
</tr>
<tr>
<td>Ceftriaxone (30)</td>
<td>10(14.9)</td>
<td>23(41.1)</td>
<td>3(12.0)</td>
<td>0</td>
<td>36(21.6)</td>
</tr>
<tr>
<td>Amikacin (30)</td>
<td>5(7.4)</td>
<td>4(7.1)</td>
<td>0</td>
<td>2(11.1)</td>
<td>11(6.6)</td>
</tr>
<tr>
<td>Nitrofurantion (300)</td>
<td>2(2.9)</td>
<td>4(7.1)</td>
<td>0</td>
<td>0</td>
<td>6(3.6)</td>
</tr>
<tr>
<td>Ciprofloxacin (5)</td>
<td>0</td>
<td>3(5.3)</td>
<td>0</td>
<td>0</td>
<td>3(1.8)</td>
</tr>
</tbody>
</table>

DISCUSSION

In present study, S. flexneri was the predominant isolated shigella species, followed by S. dysenteriae, S. boydii and S. sonnei. This is consistent with other reports from other part of Iran (3). However, temporal and spatial variations in the isolation of Shigella species have been reported in various parts of world from time to time. Before 1984, S. flexneri was the predominant species isolated sporadically from 3% of diarrhea cases in Calcutta, India (19). During 1990-1992 S. dysenteriae type 1 was isolated more from this area (20), again during 2001 to 2004 S. flexneri was the most prevalent serogroup (8). In an epidemiological study of acute bacterial diarrhea in children during 2002-2003 in Bahia, Brazil, S. sonnei was the most frequent pathogen (5). From 1999 to 2000 S. flexneri and S. dysenteriae were the most common shigella isolates in Lagos, Nigeria (21). From 1987 to 2002 S. sonnei was predominant species in central Turkey (22). In our study, S. sonnei was the least frequent isolates among other Shigella species that was in agreement with findings of Lee et al from Malaysia (23).
In our study, S. flexneri types 1b, 2a and 2b were the most common serotypes isolated from children with bacillary dysentery, respectively (Table 1). These findings are differed from other reports. In China (14) the most common serotypes of S. flexneri were 1a, X, and 2a and in Thailand (24) the three most frequently encountered serotypes were 2a, 1b and 3b, respectively. Among S. dysenteriae isolates that were serotyped in current study, types 1 and 2 were more prevalent. This finding was supported with some other studies (2,25,26). In the present study, S. flexneri and S. sonnei were mostly found in children aged 7-10 years, while S. boydii was found in children <2 years old, this was not supported with other reports (2,5). However, S. dysenteriae was mostly isolated in children aged 11-14 years old.

Our study also documents the trend of multi-resistant Shigella species associated with bacillary dysentery in Hamadan over a four-year period. Multiple antibiotic resistances were observed among the strains of Shigella isolates and more than four commonly used antibiotics were ineffective against Shigellae isolates in this study. S. flexneri showed a high degree of resistance to most of the commonly used antibiotics, such as chloramphenicol, ampicillin, co-trimoxazole, and tetracycline (table 3). This finding is in agreement with other reports from developing countries such as India (8,9), Bangladesh (10), Brazil (13), China (14), Nigeria (21) and Thailand (24). However, in our experience S. flexneri also showed resistance to nalidixic acid (44.7%) and co-amoxiclav (29.8%). These results are slightly differed from other reports (3,13,27). In developing countries and low socio-economic conditions including Iran, S. flexneri is still the predominant serotype (3,10,21,25).

Evaluating the trends in the resistance patterns of Shigella species demonstrated that S. dysenteriae is currently significantly more resistant than the other Shigella species in Hamadan, especially to the commonly used antimicrobial agents. This finding is of utmost importance since S. dysenteriae is, at present, the second predominant species in this region. In our survey, S. dysenteriae showed the highest rate of resistance to most of the tested antibiotics including ampicillin, co-trimoxazole, chloramphenicol, tetracycline, nalidixic acid, co-amoxiclav and ceftriaxone (table 3). Resistance was emerged even to more potent antimicrobial agents such as ciprofloxacin, nalidixic acid, co-amoxiclav and nitrofurantoin.

Resistance to nalidixic acid (85.7%), ceftriaxone (41.1%) and co-amoxiclav (37.5%) was not compatible with studies of some other countries such as Ethiopia (27), Israel (28), Thailand (24), Turkey (22), and Chile (13).

S. boydii and S. sonnei also showed a high level of resistance to chloramphenicol, ampicillin, co-trimoxazole, and tetracycline, but most of them were susceptible to co-amoxiclav and ceftriaxone. In children with severe shigellosis, especially in those who are hospitalized, parenteral ceftriaxone is effective and usually recommended. In our study, all shigella isolates, except S. dysenteriae, were sensitive to ceftriaxone. All isolates of S. boydii were susceptible to amikacin, ciprofloxacin, nitrofurantoin and nalidixic acid. Increasing resistance of Shigella strains to nalidixic acid has been emerged over the past few decades in some part of the worlds, especially in developing country (14,21,25,26). This may be due to inappropriate use of this drug.

In our study, all shigella isolates, except S. boydii, were resistant to nalidixic acid. In spite of the worldwide spread of resistant strains, the use of nalidixic acid is still recommended by the World Health Organization guidelines for the management of acute bloody diarrhea in children (2).

Although resistance to ciprofloxacin has been rarely reported, nearly all Shigella isolates (except a few strains of S. dysenteriae) remained susceptible to this agent. Ciprofloxacin is often recommended as empirical therapy in areas with
high resistance to Shigella. It is, however, not approved for children because of the potential risk of damage to growing cartilage (29).

In conclusion, our results revealed that multi-resistant strains of Shigella (in particular S. dysenteriae and S. flexneri) are present in Hamadan and emphasize the importance of maintaining surveillance of these strains in order to assess local susceptibility patterns and empiric therapy. Most strains of Shigella species in this study were found to be resistant to chloramphenicol, ampicillin, co-trimoxazole, nalidixic acid and tetracycline, and sensitive to ciprofloxacin, amikacin and nitrofurantoin.

REFERENCES


