Prevalence of *Shigella* Related Diarrhea in Ambo Town and Antibiotic Susceptibility of the Isolated Strains

By

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ABSTRACT

Shigellosis is a major dysenteric disease of developing countries where there are poor personal hygiene, waste disposal and water and food quality. Children are the most affected by the disease because of lower immunity. The prevalence of Shigella, the causative agent for Shigellosis, and susceptibility patterns against the commonly used antibiotics were the prior objectives. Seven out of the total 24 diarrheal stool samples (29%) collected during the study period (March to May 2011), were confirmed for Shigella sp following standard biochemical testes. About 43% isolates were obtained from children under 15 years. The antimicrobial resistance of the isolates against Ampicillin, Trimethoprim-Sulfamethoxazole, Chloramphenicol, Gentamicin and Tetracycline were 100%, 85.7%, 71.5%, 71.5% and 71.5% respectively. The antimicrobials Nalidixic acid, Norfloxacin and Ciprofloxacin were not resisted by any of the strains and their susceptibility patterns were 100%, 100% and 85.7% in their listed order. Multidrug resistance against two to five antimicrobials was also recorded. As to the recommendations for combating the disease, keeping personal hygiene, eradicating flies, using safe waste disposal method and appropriate usage of antibiotics were suggested.

Key words: antimicrobials, prevalence, resistance, Shigella, Shigellosis, susceptibility.

INTRODUCTION

Shigellosis, a dysenteric disease caused by Shigella species is more prevalent in areas of poor sanitation and hygiene in developing countries. Earlier studies from Africa, Asia and Latin America reported the susceptibility of the disease in connection with lack of sanitation (Asgharet al, 2002; Abera, 2004; Bhattacharya et al., 2005; Andualem et al., 2006; Rahbar et al., 2007). Transmission of this disease is through faecal-oral route through contaminated hands, food and water and as epidemics where there are close personal physical contacts such as refugee camps, jails and temporary public settlements (Ochei and Kolhatkar, 2000).

The disease is more prevalent among children aged between one to four years (WHO, 1999) in the tropical countries. According to Wilson et al. (2006). 71.4% S. flexneri isolates were observed with children less than five years of age in western Nepal. A similar result was found in Iran (Rahbar et al., 2007) and in Africa (Wasfy et al., 2000; Opintan and Newman, 2007) where children less than 12 years of age were the most exposed to shigellosis. In Northwest Ethiopia, 36.4% of Shigella strains were isolated from children below 15 years of age (Yismaw et al., 2006).

Even though dysentery caused by Shigella is more common, Salmonella, Morganella morganii and Vibrio cholerae and Entamoeba histolytica (Dey et al., 1999) are also some other causative agents. There are four biochemically different serotypes of the genus Shigella; S. flexneri, S. dysentriae, S. boydii and S. sonnei (Talukder et al., 2000; Wilson et al., 2006) distributed in different geographical areas with unknown reason. Several studies in Africa and Asia have shown S. flexneri and S. dysentriae are the predominant isolates (Khalil et al., 1998; Opintan and Newman, 2007; Kuo et al., 2008). Controversially, in countries like Israel and Spain, S. sonnei has been highly existent. Though small in number, the other serogroups like S.flexneri and S. dysentriae have also been isolated from these countries (Parats et al., 2000; Ashkenazi et al., 2003). In Ethiopia, S. flexneri and S. dysentriae were the major isolates of Shigella strains having 99 and 1% respectively (Belay et al., 2000). Predominance of Shigella infections were also reported (Abera, 2004; Andualem et al., 2006; Yismaw et al., 2006) from the different parts of Ethiopia.
The antimicrobial resistance pattern of the organism is reported to change (Parats et al., 2000; Rahbar et al., 2007). Increasing resistance to several antibiotics has been reported from Israel (Ashkenazi et al., 2003) and African countries (Wasfy et al., 2000; Yismaw et al., 2006; Opintan and Newman, 2007). The present study was designed to find out the prevalence and antibiotic resistance pattern of *Shigella* species in and around Ambo town (Western Addis Ababa) as there was no published data available from this part of the country.

**MATERIALS AND METHODS**

The samples for the present study were collected from patients with diarrhea admitted in Ambo Hospital and Ambo Health Center from March to May 2011. A total of 24 diarrheal stool samples were collected using sterile capped containers aseptically. Demographic data (such as age groups and sex) were also considered along with collection of the samples. The samples were transported immediately (one to two hrs after received from the patients) to Ambo University Biology laboratory using ice box and inoculated in to three enrichment media: Tetrathionate, Mannitol Selenite and Gram negative broth media (Oxoid Ltd, UK) and incubated for 24 hrs at 35°C (WHO, 1999). Subcultures have been made on Xylose-lysine-desoxycholate agar (XLD), *Salmonella-Shigella* agar (SS) and MacConkey agar media (Oxoid, UK) according to the manufacturer’s direction (Ashkenazi et al., 2003). The suspected isolates were transferred to Nutrient agar for further characterization, and morphological and biochemical tests including Gram reaction test, motility test, oxidase test, Catalase test, Triple sugar iron agar (TSI) test, Lysine iron agar test (LIA), Urease test, glucose, Mannitol and sucrose fermentation tests and H₂S production test (on LIA and TSI) (WHO, 1999).

After sub culturing and purification, pure colonies were inoculated into Triptic soy broth and incubated at 35°C for 24 hrs. The turbidity of the incubated cultures were adjusted to 0.5 McFarland standard to bring the cell density to 10⁷–10⁸ colony forming units. The 0.5 McFarland standard was prepared by mixing 0.05 ml 1.175% Barium chloride and 9.95 ml 1% sulfuric acid. Then, the Mueller Hinton agar plates were covered with the standardized broth culture by using sterile swabs. The antimicrobial disks which included Ampicillin, Chloramphenicol, Tetracycline, Trimethoprim-Sulfamethoxazole, Ciprofloxacin, Nalidixic acid, Norfloxacin and Gentamycin; from Hi-Media Laboratories, India and Oxoid Ltd, UK were placed on the surface of the plates by applying gentle pressure using sterile forceps. After incubation at 35°C for 24 hrs the diameter of the inhibition zones were measured and the results were translated as sensitive, intermediate and resistant (Andrews, 2001).

**RESULTS**

During the study period, 24 diarrheal stool samples were collected. Out of which 14(58%) were from male and 10(42%) from female patients. Of the total 50% of the samples accounted from children under 15 years of age. Seven *Shigella* strains were isolated from the total 24 diarrheal stool samples and were confirmed using standard biochemical tests, this showed 29% prevalence of the strains in the study site. Among the strains, 5(72%) were isolated from male patients and 2(28%) from females. In this regard, children below 15 years were having 43% of isolation rate of the strain.

The antimicrobial resistance patterns against Ampicillin, Trimethoprim-Sulfamethoxazole, Chloramphenicol, Gentamycin and Tetracycline were to be 100%, 85.7%, 71.5%, 71.5% and 71.5% respectively (Table 1 and Figure 1). The antimicrobials Nalidixic acid, Norfloxacin and Ciprofloxacin were not resisted by any of the strains and their susceptibility patterns were 100%, 100% and 85.7% in their listed order. Multiple drug resistance was also observed ranging from two to five drugs (Table 2 and Figure 2).
**Table 1:** Antimicrobial susceptibility patterns of isolated *Shigella* species

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>No of Shigella species (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td>T</td>
<td>5(71.5)</td>
</tr>
<tr>
<td>CN</td>
<td>5(71.5)</td>
</tr>
<tr>
<td>C</td>
<td>5(71.5)</td>
</tr>
<tr>
<td>A</td>
<td>7(100)</td>
</tr>
<tr>
<td>Cip</td>
<td>-</td>
</tr>
<tr>
<td>Na</td>
<td>-</td>
</tr>
<tr>
<td>SXT</td>
<td>6(85.7)</td>
</tr>
<tr>
<td>NOR</td>
<td>-</td>
</tr>
</tbody>
</table>

* Figures in parenthesis indicate percentages.

Key: A=Ampicillin, CN=Gentamicin, C=Chloramphenicol, T=Tetracycline, Cip=Ciprofloxacin, Na=Nalidixic acid, SXT=Trimethoprim-Sulfamethoxazole and NOR=Norfloxacin

* The key is similar to Table 1.

**Figure 1:** Antimicrobial susceptibility patterns of isolated *Shigella* species

* The key is similar to Table 1.

**Table 2:** Multiple drug resistance pattern of *Shigella* isolates

<table>
<thead>
<tr>
<th>Pattern of resistance*</th>
<th>Resistant isolates(n=7)**</th>
<th>Number of resisted antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>A,SXT</td>
<td>1</td>
<td>14.3</td>
</tr>
<tr>
<td>A,C,CN,SXT,T</td>
<td>5</td>
<td>71.4</td>
</tr>
</tbody>
</table>

* The key is similar to Table 1.

**One isolated strain didn’t show multidrug resistance; it accounted 14.3% of the total.**
DISCUSSION

The present study indicated that males were more susceptible for diarrhea (58%) than females who scored 42% of prevalence of diarrhea, which is more similar with the findings of Savadkoohi and Amadpour-kacho,(2007) in Iran, reported 56% and 44% for male and female diarrheal patients respectively. According to Huruy et al., (2008) there was 50% diarrheal prevalence in both sexes in Gondar hospital in Ethiopia.

In the present study 50% of the children under 15 years of age were admitted for diarrhea. This is in agreement with the WHO (1999) report which indicated that children under 15 years of age are most susceptible for diarrhea. The prevalence of about 29% of Shigella isolates from the affected patients in the study site indicates a higher susceptibility of the bacterium in the country. Andualem et al.(2006) reported 6.7% prevalence of the strain in Gondar which showed smaller percentage (Andualem et al., 2006).In the same study site, Gondar, Huruy, et al., (2008) and his supporters have got a 16.9% prevalence rate of the isolates which actually indicated increase in the prevalence rates of Shigella in the concerned site (Huruy et al., 2008). Besides, 3.4%, 4.04% and 7.5% prevalence rates were recorded in Eritrea, Ghana and Trinidad and Tobago respectively (Naik, 2006; Opintan and Newman,2007; Orret,2008). In contrast, one report from Awassa, Southern Ethiopia, indicated as high as 34.6% isolation rate of the strains which was thought due to the availability of suitable conditions for the transmission of the species such as unavailability of safe drinking water, poor personal hygiene and the presence of flies (Belay et al., 2000). The above possible reasons are also believed to be applicable for the current study site, Ambo town.

The high antimicrobial resistance patterns of the isolated stains against Ampicillin, Tremethprime-Sulfamethoxazole, Chloramphenicol, Gentamycin and Tetracycline which were designated as 100%, 85.7%, 71.5%, 71.5% and 71.5% respectively. For the above antibiotics from Bangladesh the resistance reported was in the order 100%, 83%, 75%, 70% and 73% respectively (Talukder et al.,2006). However, Ciprofloxacin, Nalidixic acid and Norfloxacine were not resisted by any of the isolates in the present study. The drugs Tremethoprime-Sulfamethoxazole, Chloramphenicol and Ciprofloxacin are the most commonly prescribed for shigellosis in Ambo town as the case in most other countries. However, the two drugs, Trimethoprime-Sulfamethoxazole and Chloramphenicol, were resisted by the isolates in the present observation. Probably, this is because of the continuous use of the drug by the people for other disorders without proper diagnose by physicians. Similar observations on the development of resistance of antibiotics have been reported from many parts of the country (Belay et al., 2000; Andualem et al., 2006; Huruy et al., 2008). The high rate multidrug resistance (85.7%) observed in this study is in line with the report by( Rahbar et al.,2007). This high rate of multidrug resistance and the reasons for its development needs to be established in future.
CONCLUSION

To conclude, the present study indicated high prevalence and drug resistance patterns of *Shigella* species in Ambo town. In addition, serious level multidrug resistance patterns were also recorded. These findings should be strengthened by other series of researches on the subject to appropriately survey and evaluate the prevalence and susceptibility patterns of *Shigella* species and find a solution in combating Shigellosis in Ambo town. Keeping personal hygiene, eradicating flies, providing safe drinking water, using safe waste disposal method and appropriate usage of antibiotics can reduce the risk of morbidity and mortality rate of the disease.

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REFERENCES


