Original Article

Serodiversity and Antimicrobial Resistance Pattern of Shigella Isolates at Gondar University Teaching Hospital, Northwest Ethiopia

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SUMMARY: The aims of this study were to determine the distribution of serogroups and serotypes as well as the antimicrobial resistance pattern of Shigella isolates from Gondar patients with acute diarrhea. A cross-sectional study was conducted from August 2006 to February 2008. Stool specimens were received from study subjects and cultured. Isolates were confirmed by biochemical and serological tests. The isolates were tested for antimicrobial susceptibility by the disc diffusion method. Of the 1,200 stool specimens, 90 (7.5%) yielded Shigella isolates with the serogroups of Shigella flexneri (72.2%), S. dysenteriae (10.0%), S. boydii (8.9%), and S. sonnei (8.9%). S. flexneri was the predominant serogroup. S. dysenteriae type 1 was absent, and S. sonnei was present. Eighty-five (94.5%) of the isolates showed resistance to one or more drugs, and 79% of those were multiresistant. S. flexneri showed the highest resistance (91.2%). S. flexneri serotype 1, resistant to ciprofloxacin and norfloxacin, was observed. All isolates were sensitive to nalidixic acid and ceftriaxone. Only 5 (5.6%) of the isolates were sensitive to all antibiotics tested. Based on these findings, we recommend ciprofloxacin as the drug of choice for treatment of shigellosis in Gondar, with frequent monitoring of drug susceptibility testing.

INTRODUCTION

Shigellosis is a highly infectious disease of worldwide significance: its prevalence is highest in tropical and subtropical regions of the world, where living standards are very low and access to safe and adequate drinking water and to proper excreta disposal systems are often very limited or even absent (1,2).

Four species of Shigella—S. sonnei, S. boydii, S. flexneri, and S. dysenteriae—are well-recognized human pathogens (1,3). S. sonnei and S. boydii are associated with mild illness of short duration and most often occur in developed countries (4). Infection by S. flexneri and particularly that of S. dysenteriae type 1 are very often associated with explosive epidemics, severe complications and very high mortality in developing countries (4,5).

In the past five decades, Shigella spp. have acquired plasmid-mediated multi-resistance to the commonly used first-line therapy for shigellosis (5,6). The emergence of multi-drug-resistant Shigella strains to new antimicrobial agents has become a serious problem for developing countries (6,7).

In Ethiopia, reports on Shigella spp. were very scant until a few reports from central Ethiopia appeared in 1978 and 1982 (8). Since then, many investigators, including Gedebou and Tassew (1982), showed the isolation frequency of four Shigella spp. with their resistance patterns (9).

In an analysis of specimens from in- and outpatients at Gondar Hospital, S. sonnei was reported as nonexistent (10). Later, a similar report from this area did not mention the frequency of Shigella isolates at a species level (11). Thus, to our knowledge, there is only one report on the serogroups and serotypes of Shigella isolates in the Gondar region. The present study was therefore initiated to determine the prevalence of the serogroups, serotypes, and antimicrobial resistance patterns of Shigella isolates to different antimicrobial agents in the Gondar area of northwest Ethiopia.

MATERIALS AND METHODS

This study was conducted in the Microbiology Laboratory of the University of Gondar Hospital, northwest Ethiopia, between August 1, 2006 and February 10, 2008. The study participants were patients with acute diarrhea attending the outpatient at Department of Gondar University Hospital. Patients who had been exposed to any kind of antibiotics in the past month were excluded.

Diarrheic or dysenteric fresh stool specimens were collected from study subjects for whom physicians had requested stool cultures. The specimens were immediately inoculated on MacConkey (Oxoid, Hampshire, England) and Salmonella-Shigella (SS) agar (Oxoid) and incubated at 37°C for 24 h. Oxidase negative nonlactose fermenter colonies from MacConkey and/or SS agar were picked up and inoculated in about 3 ml nutrient broth and incubated to grow for about 6 h for use in biochemical and in vitro antimicrobial susceptibility testing. Identification of Shigella was confirmed by the conventional biochemical tests, which included indole, triple sugar iron agar, urea agar, lysine iron agar, simmon’s citrate agar, motility medium, and mannitol broth. Once identified as Shigella spp. by the biochemical tests, original colonies were further tested for serogrouping and serotyping using group- and type-specific Shigella antisera following the manufacturer’s instructions (Denka-Seiken Co., Ltd, Tokyo, Japan).

The antimicrobial susceptibility testing of all strains was carried out on Muller-Hinton agar (Oxoid) with discs (Oxoid) using the single disc diffusion technique of Bauer et al. (12), against ampicillin (10 μg), tetracycline (30 μg), cotrimoxazole (125 μg), chloramphenicol (30 μg), gentami-
cin (10 μg), ciprofloxacin (30 μg), nalidixic acid (30 μg), norfloxacin (30 μg), and ceftriaxone (30 μg).

Results were read after 24 h incubation at 37°C, and the diameters of growth inhibition around the discs were measured and interpreted as sensitive or resistant according to the guidelines of the manufacturer (Oxoid). The standard reference strains of Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as controls throughout the study.

In this study, multidrug resistance was defined as simultaneous resistance to three or more antimicrobial agents, and intermediate resistance was considered sensitive.

Statistical analysis was done using SPSS version 13.0 software. Ethical clearance was obtained from the Research and Publication Office of the University of Gondar, and informed consent was obtained from the study participants and/or their guardians. The patients were managed by the attending physician following the hospital’s routine patient management system.

RESULTS

Of the 1,200 stool specimens received from the study subjects, Shigella spp. were isolated from 90 (7.5%) of them. The age distribution of Shigella-positive patients ranged from 11 months to 60 years, with a mean age of 15.1 years. Forty-eight (53.3%) of the patients who became positive for Shigella spp. were in the pediatric age group (up to 15 years). Twenty-eight (58.3%) of these patients were 0 - 5 years old. The remaining 42 (47%) were adults aged 16 years over. The ratio of females to males was 1.6:1.2.

The serogrouping and serotyping of all 90 Shigella isolates yielded serogroups A (S. dysenteriae), B (S. flexneri), C (S. boydii), and D (S. sonnei). S. flexneri formed 65 (72.2%) followed by S. dysenteriae 9 (10%), S. boydii 8 (8.9%), and S. sonnei 8 (8.9%) (Table 1). A number of serotypes were isolated in each serogroup: 9 serotypes in S. flexneri, 6 in S. dysenteriae, 6 in S. boydii, and phases I and II in S. sonnei. No isolate remained non-typable. The commonest serotypes were S. flexneri type 4 (24.6%), followed by type 2 (21.5%) and type 1 (13.9), S. dysenteriae type 2 (33.3%), S. boydii types 2 and 5 (25% each), and S. sonnei phase 1 (75%).

All the Shigella isolates in this study showed the highest resistance rates to tetracycline (90%), co-trimoxazole (84.6%), ampicillin (78.9%) and chloramphenicol (67.8%), and lowest resistance rates to gentamicin (12.2%), ciprofloxacin (2.2%), and norfloxacin (1.1%). All the isolates tested were sensitive to nalidixic acid and ceftriaxone. Only 5 (5.6%) of the total isolates were sensitive to all antimicrobial agents tested (Table 2).

Resistance to one or more antimicrobial agents was detected in 85 (94.5%) of the strains, of which 71 (79%) showed multi-resistance patterns. The predominant multiresistant patterns were ampicillin, co-trimoxazole, tetracycline (18 strains) and ampicillin, co-trimoxazole, tetracycline, chloramphenicol (40 strains).

DISCUSSION

The rate of isolation of Shigella spp. in the present study was similar to those reported by Mache et al. (13) and Ai et al. (14). However, others have reported rates as low as 3.5% and as high as 16% (15,16). The variations may be attributable to the sampling times and to differences in laboratory techniques used. A higher isolation rate (58%) was found in children 0-5 years old, which may indicate that in Gondar, shigellosis is a problem in children under the age of 5 years.

The distribution of Shigella serogroups in the present study was similar to that reported in central Ethiopia over a 30-year-period (8,17). Consistent with findings by authors from other countries (7,18-20), the present study demonstrated that S. flexneri was the predominant serogroup causing shigello-

### Table 1. Distribution of serogroups and serotypes of Shigella isolates, August 2006 to February 2008, Gondar University

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>No. (%)</th>
<th>Serotype (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B (S. flexneri)</td>
<td>65 (72.2)</td>
<td>1 (13.9), 2 (21.5), 3 (10.8), 4 (24.6), 5 (1.5), 6 (4.6), gr 3/4 (7.7), gr 6 (12.3), gr 7/8 (3.1)</td>
</tr>
<tr>
<td>A (S. dysenteriae)</td>
<td>9 (10)</td>
<td>2 (33.3), 4 (11.1), 6 (11.1), 7 (22.2), 8 (11.1), 9 (11.1)</td>
</tr>
<tr>
<td>C (S. boydii)</td>
<td>8 (8.9)</td>
<td>2 (25), 5 (25), 7 (12.5), 9 (12.5), 10 (12.5), 12 (12.5)</td>
</tr>
<tr>
<td>D (S. sonnei)</td>
<td>8 (8.9)</td>
<td>Phase I (75), Phase II (25)</td>
</tr>
</tbody>
</table>

*: Rate of the isolate.

Table 2. Percentage of antimicrobial resistance of Shigella isolates August 2006 to February 2008, Gondar University

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>% Resistance for:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>All Shigella</td>
</tr>
<tr>
<td></td>
<td>(n = 90)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>90.0</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>84.6</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>78.9</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>67.8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>12.2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2.2</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>1.1</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>0.0</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>0.0</td>
</tr>
</tbody>
</table>
sis in Gondar. Also, in line with the results found in some developing countries (2,21,22), three serotypes-type 4 followed by type 2 and type 1 combined--comprised 60% of the infections caused by *S. flexneri* (Table 1). When compared to the serogroups in Gondar over a decade ago (10), the present study showed a decrease in *S. dysenteriae* (other than in *S. dysenteriae* 1) with corresponding increases in *S. flexneri*, *S. boydii*, and *S. sonnei*.

*S. dysenteriae* type 1, the causative agent of the most serious form of bacillary dysentery to occur in many developing countries (3,4,23,24), was absent in the present study. Although it is difficult to explain its disappearance, this strain may be able to re-emerge in the future and to produce severe epidemics of disease with complications and death.

*S. sonnei*, the most commonly reported serogroup in industrialized countries (25), was reported absent in the previous study in Gondar patients (10). However, it was isolated in the present study. Gondar city, which was the seat of Ethiopian government for 200 years until the beginning of the 18th century, still attracts thousands of international tourists for its magnificent old castles, its historic significance, and its sites of interest. Thus, *S. sonnei* may have been introduced into Gondar by tourists from industrialized countries where the organism was prevalent.

The emergence and dissemination of antimicrobial resistance among *Shigella* strains are increasing global health problems that complicate the therapeutic management of severe shigellosis cases (3). *Shigella* isolates from Gondar, as in other developing countries (26-28), showed higher rates of resistance to commonly used antimicrobial agents (tetracycline, co-trimoxazole, ampicillin, and chloramphenicol). *S. flexneri* showed higher rates of resistance than the other serogroups (Table 2).

A comparison of the findings from different regions and periods, such as central Ethiopia (Addis Ababa), southern Ethiopia (Awassa), southwestern Ethiopia (Jimma), northwest Ethiopia (Gondar), and West Africa (Lagos) (Table 3), reveals that *Shigella* strains from Awassa (93%) and Lagos (90%) showed the highest rates of resistance to ampicillin. Co-trimoxazole-resistant strains also showed similar resistance rates in Lagos (85%) as in the present study (84.6%). Strains resistant to tetracycline from Awassa (90%), Lagos (79%), and Gondar (86%) in 2001-2005 and the present study (90%) also demonstrated similar higher resistance rates. During 2001-2008, chloramphenicol-resistant strains from Gondar increased from 52.8 to 67.8%, and gentamicin-resistant strains from Gondar increased from 7.9 to 12.2%.

A study reported in 1997 on Gondar inpatients by Aseffa et al. (10) also showed high resistance rates to chloramphenicol (67%), ampicillin (78%), and tetracycline (92%); medium resistance to co-trimoxazole (63%) and low resistance to gentamicin (2%). The sixfold increase in gentamicin resistance in the past 10 years (Table 3) may be due to indiscriminate overuse of the drug and related aminoglycosides by the community. However, Gizachew et al. (11) have also reported a fourfold increase in resistance to this agent. This comparative trend in antimicrobial resistance clearly indicates the serious problems of single-drug-resistant features of *Shigella* isolates in the Gondar region.

*Shigella* spp. are very well noted for their ability to rapidly develop multidrug resistance among their strains (2,10). Earlier studies in Ethiopia have reported drug resistance to as many as seven drugs (29). Aseffa et al. (10) from Gondar a decade ago reported multiple drug resistance for five commonly used antimicrobial agents. In the present study we found that 71 (79%) of the isolates are resistant to as many as five to six drugs, from which *S. flexneri* (91.2%) has the highest share. These data demonstrate the worsening situation of multiple drug resistance problems, particularly in relation to *S. flexneri* serogroups in the Gondar region.

This alarming rise in multiple drug resistance in Gondar isolates of *Shigella* against commonly used antimicrobial agents may be related to the indiscriminate overuse of antimicrobial agents in the Gondar region in the past 20 to 30 years; such overuse may have resulted in the selection of mutants or the acquisition of genes for resistance from other organisms, through a process of genetic exchange (13,19,20).

In central (13), southern (30), and southwest (31) Ethiopia, nalidixic acid was introduced to treat shigellosis that was resistant to commonly used drugs. Its overuse, however, rapidly brought about resistance to this agent. Neither the present study nor our previous one (10) found nalidixic-acid-resistant strains of *Shigella* in Gondar. This is probably because this agent was not introduced into Gondar until very recently. In Gondar, although co-trimoxazole was resistant to the first line of therapy, it continued to be prescribed for the treatment of diarrheal diseases, even up to the present day (10,11).

Fluoroquinolones (ciprofloxacin and norfloxacin) emerged in Gondar in the 1990s (verbal communication with the hospital pharmacy of the University) as the preferred agents for the treatment of shigellosis. Since then, physicians have docu-

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**Table 3**: Comparison of resistance rates of *Shigella* spp. to commonly used antimicrobial agents in different periods and different regions (Addis Ababa, Awassa, Lagos, Jimma, Gondar, and present study), Gondar University

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<tbody>
<tr>
<td>Ampicillin (10 µg)</td>
<td>70</td>
<td>93</td>
<td>90.0</td>
<td>70.1</td>
<td>79.4</td>
</tr>
<tr>
<td>Co-trimoxazole (125 µg)</td>
<td>52</td>
<td>56</td>
<td>85.0</td>
<td>32.0</td>
<td>73.4</td>
</tr>
<tr>
<td>Chloramphenicol (30 µg)</td>
<td>50</td>
<td>--</td>
<td>77.0</td>
<td>40.0</td>
<td>52.8</td>
</tr>
<tr>
<td>Tetracycline (30 µg)</td>
<td>74</td>
<td>90</td>
<td>79.0</td>
<td>63.6</td>
<td>86.0</td>
</tr>
<tr>
<td>Gentamicin (10 µg)</td>
<td>0</td>
<td>4</td>
<td>--</td>
<td>1.3</td>
<td>7.9</td>
</tr>
<tr>
<td>Ciprofloxacin (30 µg)</td>
<td>--</td>
<td>--</td>
<td>nib</td>
<td>--</td>
<td>nib</td>
</tr>
<tr>
<td>Norfloxacin (30 µg)</td>
<td>--</td>
<td>--</td>
<td>nib</td>
<td>--</td>
<td>nib</td>
</tr>
<tr>
<td>Nalidixic acid (30 µg)</td>
<td>14</td>
<td>10</td>
<td>11.0</td>
<td>6.5</td>
<td>nib</td>
</tr>
<tr>
<td>Ceftriaxone (30 µg)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>nib</td>
</tr>
</tbody>
</table>

nil: all strains are sensitive. --: not done (tested for).
mented the excellent activities of these agents. The present study, however, has revealed *S. flexneri* serotype 1, which is resistant to ciprofloxacin and norfloxacin and susceptible to nalidixic acid and ceftriaxone.

Hakanen et al. (32) have described, however, the emergence of a new quinolone resistance pattern in *Salmonella enterica*. These isolates are susceptible to nalidixic acid but exhibit reduced susceptibility to ciprofloxacin. Those authors determined the MICs of 16 such *Salmonella* isolates, and found that almost all of them were susceptible or had intermediate resistance to nalidixic acid. The disc diffusion test results for nalidixic acid also varied between susceptible and intermediate resistance. The authors concluded that the increase in isolates that were susceptible to nalidixic acid but that had reduced susceptibility to ciprofloxacin may threaten the value of the nalidixic acid disc test to screen for reduced fluoroquinolone susceptibility in salmonellosis. It was then recommended that similar strains be tested by a fluoroquinolone Etest or another suitable MIC method.

Referring to the report by Hakanen et al. (32), *Shigella* isolates from Gondar, resistant to ciprofloxacin and susceptible to nalidixic acid, have properties similar to those of *S. enteritica* isolates. Consequently, Gondar isolates should have been screened for their susceptibility to ciprofloxacin and nalidixic acid by multiple techniques before they were reported as susceptible or resistant. Unfortunately, however, there are neither Etest nor agar dilution test facilities in Gondar laboratories to determine the MIC.

Moniot-Ville et al. (33) have also described two resistance mechanisms found in a nalidixic-acid-susceptible but fluoroquinolone-resistant strain of *Escherichia coli* (Q2 [resistant strains]) selected under norfloxacin therapy. As compared with the susceptible *E. coli* (Q1 [susceptible strains]) isolated before treatment, changes in outer membrane proteins and lipopolysaccharides in *E. coli* (Q2) were associated with a 1.5- to 3-fold decrease in the uptake of fluoroquinolones but not in that of nalidixic acid. From these results, it was possible to conclude that a decrease in outer membrane permeability associated with an alteration of DNA gyrase was responsible for the unusual quinolone resistance phenotype of *E. coli* (Q2). Related to this event, it can be suggested that the mechanism of quinolone resistance in *S. flexneri* of Gondar isolates that were resistant to the fluoroquinolones (ciprofloxacin and norfloxacin) and susceptible to nalidixic acid may be the same as the one described here for *E. coli* by Moniot-Ville et al. (33).

In conclusion, the study showed that *S. flexneri* is the predominant serogroup with the highest of multi-resistant rates to antimicrobials. We also found the emergence of new *S. flexneri* serotype 1 strains resistant to the fluoroquinolones and susceptible to nalidixic acid, and showed that a multi-resistant serogroup of *S. sonnei* has been introduced into Gondar. Physicians in the region should be aware of these problems and perform drug susceptibility testing on each clinical isolate of *Shigella* using disc diffusion and agar dilution methods, and change the empiric antibiotic treatment accordingly. Further studies on the basic science behind antibiotic resistance mechanisms are required to understand the progress of antibiotic resistance in *Shigella* spp.

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