

Antimicrobial susceptibility pattern of *Shigella* isolates in Awassa

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Abstract: A prospective study was conducted in Awassa, Southern Ethiopia from July 1997 to April 1998 in order to determine the antimicrobial pattern of *Shigella* strains. Moreover, the study was intended to form the basis of treatment guidelines for *shigellosis* appropriate to local conditions. In addition, the study was designed to accumulate epidemiological information on the resistance patterns of *Shigella* isolates of public health importance. A total of one hundred *Shigella* strains were isolated from 289 cultures received at the Center for Health Research and Laboratories (CHRL), collected from different health units. Ninety-nine strains were *Shigella flexneri* group B and one *Shigella dysenteriae* group A isolated. All isolates were susceptibility tested for nine antibiotics using disc diffusion technique. The result showed that 96% and 90% strains were susceptible to gentamicin and nalidixic acid respectively. High rate of resistance was particularly documented against the following antibiotics: ampicillin (93%), erythromycin (90%), and tetracycline (90%). Fifty-six percent of *Shigella flexneri* Group B isolates were found to be resistant to trimethoprim-sulfamethoxazole, which is one of the drugs of choice for treating shigellosis in Ethiopia. Multiple drug resistance to as many as six antibiotics, i.e. ampicillin, cephalotin, chloramphenicol, erythromycin, tetracycline and trimethoprim-sulfamethoxazole has been observed in this study. One strain of *Shigella flexneri* was found to be resistant to eight drugs. The findings are discussed and recommendations forwarded. [*Ethiop. J. Health Dev.* 2000;14(2):149-154]

Introduction

The term shigellosis refers to an acute inflammatory reaction of the intestinal tract caused by bacteria of the genus *Shigella*. *Shigella* has a worldwide distribution (1) and is of special concern in developing countries. In Ethiopia, *Shigella dysenteriae* and *Shigella flexneri* account for about 80% of isolates (2, 3, 4).

Reports from the Southern Nations, Nationalities and Peoples Health Bureau (SNNPHB) indicated that in 1995/6 diarrhoea was among the top ten causes of morbidity (5). Out of the total 182,122 outpatient visits in the region, 6195 cases were for bloody diarrhoea and 5045 were for watery diarrhoea.

Moreover, diarrhoea is one of the leading illnesses recorded as discharge diagnosis at the hospitals in the region. Out of 31,259 discharges 57(3.0%) were due to diarrhea. Diarrhoea was reported among the top 10 leading causes of death in hospitals. Out of 1730 hospital deaths 57 (3.3%) were due to diarrhoeal diseases (5).

Multiple drug resistance of *Shigella* was first noted in Japan in the 1950's (6). A *Shigella* strain, which caused an epidemic with thousands of deaths in Central America (7), was found to be resistant to chloramphenicol, streptomycin, sulfadiazone and tetracycline.

A study by Guyot (8) in Monrovia, Liberia, showed that more than 80% of the strains of *Shigella* isolates were resistant to ampicillin, cotrimoxazole and tetracycline and 65% were resistant to chloramphenicol. The antimicrobial susceptibility patterns for 30 *Shigella* isolates

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in Cordoba, Argentina showed that most strains were resistant to multiple antibiotics (9).

S. dysenteriae epidemics caused by multiple drug resistant strains have also been reported from Sri Lanka and Bangladesh (10, 11).

Of 232 strains of *Shigella* isolated in Leningrad and the Leningrad Region (12) it was reported that 95% of the isolates were resistant to one or more relevant antibiotics. The strains were most commonly resistant to streptomycin (94%) and tetracycline (91.8%). An observation made by Afeworki in Ethiopia (2) indicated that 84% of *Shigella dysenteriae* type 1 and 65% of type 3 were resistant to five drugs: ampicillin, chloramphenicol, streptomycin, sulfadiazone and tetracycline. A study done at Ethio-Swedish Children's Hospital in Addis Ababa observed multiple drug resistance (4). Over 80% of *Shigella* isolates were found to be resistant to streptomycin, tetracycline, and trimethoprim-sulphamethoxazole. Nearly 75% were reported to be resistant to ampicillin and chloramphenicol, 74% to sulphadiazine. Resistance to cephalotin was close to 25% while gentamicin or kanamycin resistance was under 10% (4).

Retrospective analysis of *Shigella* isolates collected over a two and half-year period in Awassa, Ethiopia showed multiple drug resistance. Of 179 strains of *Shigella* 81.5% were resistant to tetracycline, 67.6% to ampicillin and 70.9% to erythromycin; whereas they were mainly susceptible to gentamicin and kanamycin, (unpublished observations). Based on this information the present study was undertaken to obtain more reliable data on antibiotic susceptibility of *Shigella* strains isolated in Awassa, Southern Ethiopia.

Methods

Study site and Subjects: This prospective study was carried out between July 1997 and April 1998 in Awassa, which is located 275 km.

South of Addis Ababa. Bushulo Health Center, Dilla Hospital, Yirgalem Hospital, Lekku Health Center and Wollayita Sodo Hospital were sample collection sites. A questionnaire for gathering information including on age, sex, address, patient code number and laboratory result report forms were used to collect data.

The study subjects were male and female (both adults and children). Patients coming to health units complaining of diarrhoea that contained blood were eligible for inclusion into the study. Other criteria were:

- a. onset of illness less than five days prior to first visit.
- b. a patient must not have received antibiotic treatment for this episode
- c. verbal consent to give specimen

Specimen collection and transportation: Two swabs of stool specimen were unnecessarily collected from the cases selected. The swab was immediately inserted into the transport media, pushed to the bottom of tube and the procedure was repeated with the second swab.

The top parts of the sticks were broken off and the screw top was firmly tightened. Each test tube with a specimen was labeled and immediately refrigerated. Refrigeration was achieved during transportation in a well-insulated box with frozen refrigerated packs.

The collected specimens were stored and transported to Awassa Center for Health Research & Laboratory (CHRL).

Stool was tested for the presence of *Shigella*. Transportation and preservation of specimen was done by using Cary-Blair (BBL) media. MacConkey agar (DIFCO), Salmmonella-Shigella agar (DIFCO) and Xylose Lysine Deoxycholate agar (DIFCO) were used for the isolation of the organism. Serotyping was determined by Kligler Iron agar (DIFCO). Muller Hinton agar (DIFCO) was used for susceptibility testing and all antibiotic susceptibility discs used were from OXOID,

England.

Microbiological methods: Specimens collected in Awassa and transported from other sites were immediately inoculated onto plates containing MacConkey agar (DIFCO), Salmonella-shigella agar (DIFCO) and a selective media Xylose Lysine Deoxycholate agar (DIFCO). All plates were incubated at 35°C - 37°C for 18-24 hours and examined for the presence of *Shigella* (major laboratory steps based on CDC & WHO standard guidelines). The isolates were biochemically identified following the methods of Edwards and Ewing (13), and then sero typed by slide agglutination using commercial antisera (DIFCO).

Reference strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, were tested regularly as controls according to the National Committee for Clinical Laboratory Standards (NCCLS) (14).

To determine sero-types Agglutination tests were carried out on a clean glass slide using a straight wire for removing a portion of the growth from the surface of Kligler Iron agar (KIA) (DIFCO) and emulsifying in saline. Agglutination reactions were observed with the naked eye over a fluorescent light source.

Drug susceptibility tests for each *Shigella* isolate was carried out according to the agar disc diffusion method (15) and included the following antimicrobial agents: Tetracycline (T) trimethoprim-sulfamethoxazole (TSM), erythromycin (E), ampicillin (A), kanamycin (K), gentamicin (G), nalidixic acid (Na), chloramphenicol 8 and cephalothin (Cf).

Results

Two hundred and eighty nine specimens were collected for culture and sensitivity testing. One hundred isolates of *Shigella* were found. Of the isolates, 99% were *Shigella flexneri* group B and 1% *Shigella dysenteriae* group A.

Table 1 shows antimicrobial susceptibility patterns for the *Shigella* isolates. One strain of *Shigella dysenteriae* group A was found to be susceptible to three drugs namely cephalothin, gentamicin and kanamycin. This strain was resistant to ampicillin, chloramphenicol, erythromycin, nalidixic acid, trimethoprim-sulfamethoxazole and tetracycline. The isolated strains of *Shigella flexneri* group B were susceptible to gentamicin (96%) nalidixic acid (90%) and kanamycin (86%). The highest prevalence antibiotic resistance was documented to be against ampicillin (93%) followed by erythromycin (90%) and tetracycline (90%). Fifty six percent *Shigella flexneri* group B isolates were resistant to trimethoprim-sulphamethoxazole. Multiple drug resistance was observed in this study ranging from two to eight drugs (Table 2).

Table 1: In vitro antimicrobial susceptibility pattern of *Shigella* isolates Awassa July 1997-April 1998. N = 100

Type of antibiotic	Susceptible (%)	Intermediate (%)	Resistant (%)
Gentamicin	96	2	2
Nalidixic acid	90	-	10
Kanamycin	86	6	8
Cephalothin	53	8	39
Trimethoprim-Sulfamethoxazole	44	-	56
Chloramphenicol	34	3	63
Tetracycline	10	-	90
Erythromycin	2	8	90
Ampicillin	7	-	93

Table 2: Multiple drug resistance patterns occurring in *Shigella* isolates Awassa Southern Ethiopia. July 1997-April 1998

Antibiotic	Percent of resistant isolates
A, T	82
A, C, TSM	59
A, TSM	56
A, TSM, T	43
A, TSM, T, C	27
A, C, E, TSM, T	11
A, C, Cf, E, TSM, T	10
A, C, E, K, Na, TSM, T	1
A, C, Cf, E, K, Na, TSM, T	1

Key: A = ampicillin, E = chloramphenicol, Cf = cephalothin, T = tetracycline, K = kanamycin, E = erythromycin, Na = nalidixic acid

In this study 48 out of 100 patients with *Shigella* and 92 out of 189 patients (49%) without *Shigella* visited a health care providing unit by the second day of the onset of diarrhoea. The highest number of isolates, 23% were obtained in the dry months of December 1997, 19% January 1998 and 18% February 1998. The lowest number of isolates 1% was obtained in the wet month of July 1997.

Discussion

In this study of acute bloody diarrhoea *Shigella dysenteriae* group A was observed only in one case (1%). The predominance of *Shigella flexneri* in developing countries has been previously reported (11, 21). In Ethiopia, the dominance of *Shigella flexneri* has been observed in several studies (3, 4, 16, 17, 18). The present study in Awassa confirms this.

Of the ninety-nine *Shigella flexneri* group B strains isolated, 95 were found to be susceptible to gentamicin and 90 to nalidixic acid. Nalidixic acid, is not a widely used drug in the Southern Region of Ethiopia for the treatment of *Shigellosis*. Trimethoprim-sulfamethoxazole is however a widely used antibiotic in this Region.

Fifty-five (55.5%) of *Shigella flexneri* strains were observed to be resistant to trimethoprim-sulfamethoxazole in this study. Other studies also showed that a few trimethoprim-sulfamethoxazole resistant strains of *Shigella flexneri* had been isolated in Ethiopia (19, 20). In contrast, Admasu and Geydi observed that *Shigella flexneri* strains were uniformly sensitive to trimethoprim-sulfamethoxazole (16). In other countries, trimethoprim-sulfamethoxazole resistant type 1 has been reported to exceed 25% (11). In Thailand 42-43% (21) and USA 7% TSM resistant *Shigella dysenteriae* types were found (22).

The antimicrobial susceptibility of *Shigella* is decreasing and the increase of trimethoprim-sulfamethoxazole resistant strains is reaching a

worrying level in the Southern Region of Ethiopia. The fact that this drug is available in the open market and often taken without medical advice may have contributed to the situation.

In the present work, multiple drug resistance to as many as 6 (A, Cf, C, E, TSM, T) antimicrobials was observed. Similarly, it was found that multiple drug resistance (T, C, A, Cb, S, Su) of *Shigella flexneri* was present in other studies from Ethiopia (4, 16, 18, 19) as well as in other countries (8, 9, 12, 23, 24). One strain of *Shigella flexneri* that we isolated was resistant to as many as 8 drugs (A, C, E, Na, TSM, T, K, Cf).

The problem of shigellosis is especially acute where general hygiene and environmental sanitation are poor and where there is inadequate supply of water as well as unsafe water. Lack of laboratory facilities in many health units has led to wrong diagnosis of bacillary dysentery as amoebiasis. Failure of early case detection and indiscriminate use of antibiotics may have played a role in development of drug resistance and thus made treatment ineffective.

Continuous surveillance of drug resistant *Shigella* is recommended. Moreover, antibiotic treatment should be managed solely on clinical grounds and drug sales must be only on prescription basis. It is important that drugs like nalidixic acid be available only in mandated health units.

Finally, public health measures, such as improving personal and domestic hygiene (hand washing and avoiding flies) and intensive health education aimed at prevention and control of diarrhoeal disease should be conducted regularly and safe water supply and proper waste disposal must be implemented.

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