Prevalence and antimicrobial susceptibility patterns of *Shigella* species in Asmara, Eritrea, northeast Africa

Durgadas Govind Naik

Department of Clinical Laboratory Science, College of Health Science, University of Asmara, Asmara, Eritrea

Background and Purpose: Diarrheal diseases are an important cause of morbidity and mortality in children in developing countries. Of the bacterial causes of dysentery, *Shigella* are the major enteropathogens with outbreak potential and common development of antimicrobial resistance. This study determined the prevalence and antimicrobial susceptibility patterns of different spp. of *Shigella* in Asmara, Eritrea.
Methods: Diarrheic stool specimens of a total of 2420 children were screened for *Shigella* organisms over a period of 3 years using standard methods. The *Shigella* isolates were tested for antimicrobial susceptibility pattern by the disk diffusion method.
Results: Of the 84 *Shigella* isolates, 54 were *Shigella flexneri* and 20 were *Shigella dysenteriae* type 1. High rates of resistance were observed against ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole; 6% of the *S. flexneri* isolates were resistant to nalidixic acid.
Conclusion: The study emphasizes the need for continuous monitoring of the occurrence of *Shigella* organisms and their antimicrobial susceptibility pattern for the successful treatment and control of *Shigella* dysentery, and also for the development of public health policy for populations at risk for shigellosis.

Key words: Diarrhea, dysentery, microbial sensitivity tests, prevalence, *Shigella*

Introduction

Diarrheal diseases are an important cause of morbidity and mortality worldwide. Of these, dysentery plays a major role in morbidity and mortality, particularly in children in developing countries. Among the bacterial causes of dysentery, *Shigella* spp. continue to be the most important, with a high infectivity rate and the development of antimicrobial resistance. The infective dose for *Shigella* dysentery is very low. Few bacilli (200) are sufficient to initiate infection [1,2]. Four *Shigella* spp. are recognized human pathogens — *Shigella dysenteriae, Shigella flexneri, Shigella sonnei, and Shigella boydii.* Of these, *S. sonnei and S. boydii* are associated with mild illness of short duration. Infection by *S. flexneri* is generally more severe and lasts longer. *S. dysenteriae* type 1 causes the most severe illness associated with complications and high mortality. In Central America, the *S. dysenteriae* type 1 epidemic began in 1968, and during a 10-month period, 112,000 cases with 8300 deaths were reported from Guatemala. In most of the developing countries, *S. flexneri* is the leading cause of shigellosis, particularly in an area of limited environmental health control. Reiter's syndrome is a late complication of *S. flexneri* and hemolytic uremic syndrome of *S. dysenteriae* type 1. *Shigella* epidemics have been reported from different parts of the world [3,4]. In Eritrea, diarrheal diseases play a major role in morbidity in children. The present study was undertaken to determine the prevalence and the antimicrobial susceptibility patterns of *Shigella* serotypes in Asmara, Eritrea.

Methods

The study was carried out in the Department of Microbiology of the Central Health Laboratory, Asmara, Eritrea — the national reference health laboratory. The

Corresponding author: Durgadas Govind Naik, Department of Clinical Laboratory Science, College of Health Science, University of Asmara, Asmara 9582, Eritrea.
E-mail: durga_das2002@yahoo.com

study period extended from January 2000 to December 2002. Fresh single stool specimens were collected in sterile, disposable containers from each patient included in the study. In case of possible delay, Carry-Blair transport medium was used. Standard procedures were followed to isolate, identify, and confirm *Shigella* isolates [5,6].

In brief, stool specimens were processed within 1 h of collection. For optimal isolation of *Shigella*, two different selective media were used; a general purpose medium with low-sensitivity MacConkey agar and a high-selectivity xylose lysine desoxycholate (XLD) medium (BD Microbiology Systems, Sparks, MD, USA and Oxoid Limited, Hants, England) were used. A single drop of liquid stool specimen was plated on MacConkey and XLD media, and incubated at 37°C for 24 h. Typical colorless colonies on MacConkey agar and red colonies on XLD were subcultured on Kligler iron agar.

The isolates that gave negative results for gas production and hydrogen sulfide production were further tested for motility and urease production. The non-motile, urea-negative organisms were serotyped by slide agglutination using *Shigella* antisera. Serotyping was performed using polyvalent and monovalent *Shigella* antisera obtained from Becton Dickinson (BBL; Becton Dickinson Microbiology Systems, Cockeysville, MD, USA), and Sanofi Diagnostics Pasteur (Marnes-la-Coquette, France), respectively.

The confirmed isolates were subjected to the antimicrobial susceptibility test by disk diffusion method as described by the National Committee for Clinical Laboratory Standards document [7]. Mueller-Hinton agar, poured to a uniform depth of 4 mm, was used for susceptibility testing. Each culture to be tested was streaked on tryptone soy agar to obtain isolated colonies. After overnight incubation at 37°C, 4 to 5 isolated colonies were lightly scooped with an inoculating loop and transferred to a tube containing 5 mL of sterile normal saline and vortexed thoroughly. The bacterial suspension was compared with 0.5 McFarland turbidity standard, which gives 1 to 2×10^8 colony forming units per mL. Within 15 min after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the suspension. The swab was rotated several times and then pressed firmly against the inside wall of the tube just above the fluid level to remove excess inoculum. The swab was streaked 3 times over the dried surface of Mueller-Hinton agar, while rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculum. Finally,

the rim of the agar was swabbed. The antimicrobial disks that were stored in the refrigerator were brought to room temperature and dispensed onto the surface of the inoculated agar plate using a disk-dispensing apparatus. This was done within 15 min of inoculation. Each disk was gently pressed down to make contact with the agar surface. After the disks were placed, the plates were inverted and incubated at 35°C for 18 h.

After incubation, the diameter of the zones of complete inhibition (including the diameter of the disk) was measured in millimeters using a ruler. The zones of growth inhibition were compared with the zone-size interpretative table and recorded as susceptible, intermediate, or resistant to each drug tested. To verify that antimicrobial susceptibility test results were accurate, *Escherichia coli* American Type Culture Collection 25922 quality strain was used. The antimicrobial disks were obtained from Becton Dickinson Microbiology Systems. A total of 5 antimicrobials — ampicillin, chloramphenicol, ciprofloxacin, trimethoprim-sulfamethoxazole, and nalidixic acid — were tested against *Shigella* isolates.

Results

From January 2000 to December 2002, stool samples from a total of 2420 children (less than 14 years of age) were screened for *Shigella*. In all, 84 samples from 53 male and 31 female patients yielded *Shigella* organisms. Of these, 40, 21 and 23 isolates were obtained from children below 5-, 10- and 14-years old, respectively. Thirty-eight isolates were found during the rainy season (July to September), followed by 24 isolates during the summer (February to June), and 22 isolates during the winter (October to January). The numbers of isolates for the year 2000, 2001, and 2002 were 62, 11, and 11, respectively. The distribution of the *Shigella* spp. is given in Table 1. *S. flexneri* accounted for 64% of the isolates. *S. boydii* was not isolated during the study.

All the isolates were found to be susceptible to ciprofloxacin and highly sensitive to nalidixic acid.

Table 1. Distribution of *Shigella* serogroups

Shigella serogroup	Total isolates No. (%)
Shigella dysenteriae type 1	20 (24)
Shigella dysenteriae other than type 1	8 (10)
Shigella flexneri	54 (64)
Shigella sonnei	2 (2)
Total	84 (100)

Table 2. Antimicrobial susceptibilities of the isolates

Antimicrobials	*Shigella dysenteriae* type 1 (n = 20) No. of sensitive isolates (%)	*Shigella flexneri* (n = 54) No. of sensitive isolates (%)
Ampicillin (10 µg)	1 (5)	12 (22)
Chloramphenicol (30 µg)	2 (10)	18 (33)
Ciprofloxacin (5 µg)	20 (100)	54 (100)
Trimethoprim-sulfamethoxazole (25 µg)	1 (5)	10 (19)
Nalidixic acid (30 µg)	20 (100)	51 (94)

High resistance rate was observed against ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (Table 2).

Discussion

Shigella dysentery forms a significant proportion of diarrheal diseases. The serogroup prevalence of *Shigella* organisms varies with geographic region and time of year. *S. flexneri* is the predominant sp. in the majority of the developing countries followed by *S. dysenteriae* and *S. sonnei*, whereas *S. boydii* does not appear to be a significant sp. of *Shigella* as a causative agent of shigellosis [8-10]. The presence of *S. dysenteriae* is an indicator of probable future outbreaks. The level of resistance to the commonly used antimicrobials — ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole — was quite high. Similar observation was made in other studies conducted elsewhere in the world [9,10,15]. Nalidixic acid is widely used to treat shigellosis as there is wide prevalence of resistance to commonly used antimicrobials such as ampicillin and trimethoprim-sulfamethoxazole. In the present study, 3 of the *S. flexneri* isolates developed resistance to nalidixic acid, and in such cases, ciprofloxacin can serve as a suitable alternative. In other studies, overall resistance to nalidixic acid and ciprofloxacin was quite low [9-14]. However, high rates of resistance to ciprofloxacin (62.5% *S. dysenteriae* type 1 isolates) and nalidixic acid (46% of *S. flexneri* and 100% of *S. dysenteriae* type 1 isolates) has been reported [16]. Progressive development of resistance to nalidixic acid was observed in Bangladesh, with 0% resistance in 1985 increasing to 100% resistance in 1997 [17,18].

S. flexneri was found to be the prevalent sp. in the geographic area included in this study. The presence of *S. dysenteriae* type 1 indicates potential danger of outbreaks. The determination of the antimicrobial susceptibility pattern not only helps in the successful treatment of the patient but also assists in the development of a public health policy for populations at risk for shigellosis. In conclusion, considering the progressive development of resistance to commonly used antimicrobials, this study emphasizes the need for continuous monitoring of the occurrence of *Shigella* organisms and their susceptibilities to antimicrobials.

Acknowledgments

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