High Level of Antimicrobial Resistance in *Shigella* Species Isolated From Diarrhoeal Patients in University of Gondar Teaching Hospital, Gondar, Ethiopia

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Summary

Diarrhoeal diseases are the major causes of morbidity and mortality in developing world. Shigellosis is endemic throughout the world where it is held responsible for some 165 million cases of severe dysentery. The devastating majority of these cases occur in the developing countries. The present study was conducted to determine level of antimicrobial resistance among *Shigella* species and their occurrence in 384 diarrhoeal patients, in The University of Gondar teaching hospital, Gondar, Ethiopia from August 2005 to December 2005. A pre-designed and structured questionnaire was used to collect socio-demographic and relevant clinical data of the patients. Stool samples were collected from all patients and processed for culture and antimicrobial susceptibility testing following standard procedures. *Shigella* species were isolated from 16.9% (65/384) of the patients. Resistance to tetracycline, ampicillin, cotrimoxazole, chloramphenicol, gentamicine and ciprofloxacin, respectively, was found in 87.7%, 81.5%, 75.4%, 50.8%, 10.7% and 9.2% of the isolates. Fifty nine (90.8%) of the *Shigella* species were resistant to one or more antimicrobials. Multiple drug resistance was observed in 53 (81.5%) of the isolates. The *Shigella* isolates showed high level of single and multiple resistances to the commonly used antimicrobial agents. These indicate the need for surveillance of antimicrobial susceptibility patterns in order to monitor the emergence and spread of multidrug resistant *Shigella* species.

Introduction

Diarrhoeal diseases are the major causes of morbidity and mortality in developing world (1). *Shigella*, a bacilli that belongs to the family Enterobacteriacea, is a small, non capsulated, non-motile gram negative rod and is the most common cause of bacillary dysentery which is also termed as shigellosis (2). Shigellosis is endemic throughout the world where it is held responsible for some 165 million cases of severe dysentery. The devastating majority of these cases occur in the developing countries. More than one million people are estimated to die from S*higella* infection each year. Since the late 1960s, pandemic waves of *Shigella* dysentery have strike sub-Saharan Africa, Central America and East Asia (3). In Ethiopia, like other developing countries, shigellosis is the common cause of morbidity and mortality, particularly in children (4). Unlike other secretory diarrhoeas, shigellosis is the result of invasion of the distal small bowel and/or colon by *Shigella* species (5). Infections caused by *Shigella* species are associated with symptoms which range from abdominal pain, cramps, fever, vomiting to bloody mucoid diarrhea (1).

The emergence of antibiotic resistance among bacteria is posing serious problems in antimicrobial therapy globally. The incidence varies with the area of isolation of the bacterial strain (6, 7). The progressive increase in antibiotics resistance among enteric pathogens in developing countries is also becoming a critical area of concern (8). Antibiotics therapy for shigellosis reduces the duration and severity of the disease and can also prevent potentially lethal complications. However, over the past few decades *Shigella* species have become progressively resistant to most of the first-line drugs used and the prevalence of multi drug resistant strains is an important concern of treatment (8).

In Ethiopia, Shigellosis and the emergence of antimicrobial resistant *Shigella* species is a major health problem (9). A few studies conducted previously in the country indicated high rate of resistance to commonly used antimicrobial agents such as ampicillin, tetracycline, cotrimoxazole, chloramphenicol among the isolates (10, 11, 19, 26). Since the prevalence and pattern of resistance of *Shigella* species in the country varies from one area to another, an updated information on their resistance patterns is very important for the proper selection and use of antimicrobial agents in a setting. Therefore, this study was aimed at determining the prevalence of *Shigella* species in stool samples of diarrheic patients and their antimicrobial resistance pattern in University of Gondar teaching hospital, Gondar, Ethiopia.

Patients and Methods

Study design, period and area: This cross sectional study was conducted in University of Gondar teaching hospital, Gondar, Ethiopia from August 2005 to December 2005. The hospital is a tertiary level teaching and referral hospital rendering health services for over 4 million rural and urban inhabitants in Northwest Ethiopia.

Patients and data collection: All patients who visited the outpatients and inpatients service with diarrhea, (passage of three or more loose stools per 24 hours (17), and volunteered to participate in the study were included. Considering, a 95% confidence interval, 50% proportion and 5% margin of error, a total of 384 patients were enrolled in the study. A structured questionnaire was utilized to collect socio-demographic characteristics and relevant clinical data of the patients (such as abdominal pain, vomiting, dehydration, fever, type of diarrhea).

***Sample collection and bacterial identification*:** Stool specimens from diarrheic patients were collected following standard procedure (12). The stool specimens were then inoculated on Salmonella-Shigella agar (Oxoid, Germany) plates and incubated at 35-37^{o}C aerobically for 24 hours. The plates were inspected for the presence and absence of visible bacterial colonies (12, 13). The presence of non-lactose fermenting (NLF) colonies was taken as a presumptive diagnostic tool for *Salmonella* and *Shigella*. The NLF colonies were further tested through a series of biochemical tests which included triple sugar iron agar, indole, Simmon's citrate agar, lysine decarboxylase, urea, and motility tests to identify Shigella and Salmonella species.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing of the *Shigella* and *Salmonella* isolates was done on the Muller-Hinton agar (DIFCO) against ampicillin (AMP, 30µg), tetracycline (TTC, 30µg), cotrimoxazole (SXT, 25µg), gentamicin (GEN, 10µg), chloramphenicol (CAF, 30µg) and ciprofloxacin (CIP, 5µg) following the single disc diffusion technique (14). The diameters of inhibition zones were measured and interpreted as sensitive or resistant following the manufacturers instructions (DIFCO). The standard reference strains, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, were tested as controls on the biochemical tests and antimicrobial susceptibility testing. Multidrug resistance was defined as a simultaneous resistance to three or more commonly used antimicrobial agents (15, 16).

Data analysis: The data was entered and analyzed using SPSS version 15 statistical package.

Ethical issues: The study was conducted after obtaining institutional ethical clearance from Research and Publication Office of the University of Gondar.

Informed consent was obtained from the study subjects and/or guardians. The patients were treated and managed as per the routine clinical management system of the hospital.

Results

Out of the 384 diarrheic patients included in the study, 50% were females. The mean±SD age of the study subjects was 20.9±15.8 years, and 23.7% were in the age group less than 5 years. Over 65% of the subjects used water from pipes, and 55.7% did not wash hands after toilet and before meal. Over three fourth of the participants were presented with abdominal pain. Severe dehydration and some sign of dehydration were seen in 10.4% and 37.8% of the patients, respectively. About a fifth of the patients had fever, and 38% had vomiting. The clinical characteristics of the patients are summarized in table 1.

Sixty five *Shigella* species and four S*almonella* species were isolated from the stool samples which makes their isolation rate 16.9% and 1.04%, respectively. Among the *Shigella* isolates, resistance to TTC, AMP, SXT, CAF, GEN and CIP, respectively, was observed in 87.7%, 81.5%, 75.4%, 50.8%, 10.7% and 9.2% (Table 2). The four isolates of *Salmonella* were susceptible to all antibiotics tested.

Eleven resistance patterns were observed in the *Shigella* species against the six antibiotics tested. Resistance to AMP, SXT, TTC and CAF was observed in 37.3% of the isolates which was followed by AMP, SXT and TTC (35.6%). About six percent of the *Shigella* isolates were found to be resistant to all the antibiotics tested. Resistance to one or more antibiotics was found in 90.8% of the *Shigella* isolates. The antibiotic resistance pattern of the isolates is illustrated in Table 3.

Table 1: Clinical characteristics of diarrheic patients in University of Gondar teaching Hospital, Gondar, Ethiopia, August – December 2005.

Characteristics		Frequency	Percent
Abdominal pain	Yes	296	77.1
	No	88	22.9
Dehydration	Some sign of dehydration	145	37.8
	Severe sign of dehydration	40	10.4
	No sign of dehydration	199	51.8
Type of diarrhea	Acute	223	58.1
	Watery	123	55.2
	Bloody	73	32.7
	Mucoid	27	12.1
	Persistent	161	41.9
Vomiting	Yes	146	38.0
	No	238	62.0
Fever	Yes	304	79.2
	No	80	20.8

Table 2: Resistance pattern of *Shigella* species for commonly used antibiotics in University of Gondar teaching hospital, Gondar, Ethiopia, August - December 2005.

Antibiotics	Resistance (%)
Tetracycline	57(87.7)
Ampicilllin	53(81.5)
Co-trimoxazole	49(75.4)
Chloramphenicol	33(50.8)
Gentamycin	7(10.7)
Ciprofloxacin	6(9.2)

Table 3: Multidrug-resistance pattern of *Shigella* isolates in University of Gondar teaching hospital, Gondar, Ethiopia, August – December 2005.

Resistance pattern*	Total (%)
Resistance to 3 antibiotics	
CAF-SXT-TTC	1(1.5)
AMP-CAF-TTC	2(3.1)
AM-SXT-TTC	21(32)
Resistance to 4 antibiotics	
AMP-CAF-SXT-TTC	22(33.8)
Resistance to 5 antibiotics	
AMP-CIP-GEN-SXT-TTC	3 (4.6)
Resistance to 6 antibiotics	
AMP-GEN-SXT-TTC-CAF-CIP	4(6.2)

*: AMP: ampicillin; GEN: gentamycin; SXT: cotrimoxazole; CAF: chloramphinicol; TTC: tetracycline; CIP: Ciprofloxacin

Discussion

In the present study, the prevalence of *Shigella* species among diarrhoeal patients at University of Gondar teaching hospital was 16.9%. This is a lower figure compared to a 34.6% prevalence reported in Awassa, Southern Ethiopia (19). The difference could be due to the nature of the municipal water supply system in the area which is generated from Lake Awassa and believed to be more contaminated than the municipal water supply system of Gondar town which is surface water system. A report from Iran by Savadkoohi *et al* showed a 14.05% prevalence of *Shigella* species (18). Shigellosis causes a spectrum of clinical features ranging from mild to severe and fatal diseases (9). In line with this, participants of the present study had abdominal pain (77%), severe dehydration (10.4%), some sign of dehydration (37.8%), fever (79%) and vomiting (38%).

The 1.04% prevalence of *Salmonella* species in diarrheic patients of the present study is low. This is in line with studies from developing countries by WHO (20) and Guerrant *et al* (21). An earlier study in Northwest Ethiopia showed a 2.6% prevalence of *Salmonella* species in stool samples of diarrheic patients (28).

Shigellosis is becoming an increasingly significant public health problem due to the development of multiple antimicrobial resistances, frequently resulting in treatment failure, leading in turn to health complications and deaths (22). In the present study, 90.8% of the *Shigella* species were resistant to one or more antibiotics and 81.5% of the *Shigella* species tested exhibited multiple resistances to up to six antimicrobials agents. The high resistance rate of *Shigella* isolates observed in this study is nearly in agreement with a study from Vietnam where 78.6% of the isolates were found to be multidrug resistant (23).

In addition, S*higella* isolates from Sudanese patients with diarrhoea demonstrated high resistance rates against the commonly used antimicrobial agents (AMP, TTC, SXT and CAF) (25) which is in line with the findings of the present study where a significant majority of the isolates showed resistance against TTC (87.7%), AMP (81.5%) and SXT (75.4%). This could be partly associated with the widespread use of these antimicrobials in the population for therapy since the drugs are relatively cheap and easily available (24). It can be also explained by the indiscriminate antimicrobial usage by the health care workers in the region since culture and sensitivity testing of clinical specimens is available only in a few setups (24, 26). The findings of the current study support previous studies on antimicrobial resistance of *Shigella* isolated in Ethiopia and elsewhere (8, 18, 19, 22, 27, 28, 29).

Our findings showed that 87.5%, 81% and 74.5% of the *Shigella* isolates were resistant to TTC, AMP and SXT, respectively. This is in agreement with report by Roma *et al* (19) from South Ethiopia where 90% of the *Shigella* isolates were resistant to TTC. Ashkenazi *et al* reported resistance against TTC and AMP, respectively, in 87% and 81% of *Shigella* isolates in Israel (27, 29). Resistance against SXT was reported by Assefa *et al* and Savadkoohi *et al* in 72% (18) and 73.8% (29) of *Shigella* isolates, which is in line with our finding. In the present prospective study, relatively high rate of resistance to GEN (10.7%) and CIP (9.2%) were observed as compare to previous studies conducted in different parts of Ethiopia and elsewhere (25, 19, 27, 28, 29). The increased resistance may reflect the indiscriminate and widespread uses of these antimicrobials in public health practices since people have easy access to various antimicrobials and can purchase them with out prescription (25).

In conclusion, the present study showed high isolation rate of *Shigella* species in the region, which might be a reflection of unsafe and inadequate water supply system, improper human waste disposal and management system, and poor personal hygiene. Moreover, the isolated *Shigella* species showed high level of single and multiple antimicrobial resistances to the commonly used antibiotics in the area which may reflect the indiscriminate and widespread uses of antimicrobials in public health practices in particular and the irrational use of antibiotic in general. These designate the need for a continued surveillance of antimicrobial susceptibility patterns in order to monitor the emergence and spread of multidrug resistant *Shigella* species.

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