Original article

A five-year antimicrobial resistance pattern observed in *Shigella* species isolated from stool samples in Gondar University Hospital, northwest Ethiopia

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Abstract

Background: Shigellosis is a global human health problem and an important public health problem, especially in developing countries, where substandard hygiene and unsafe water supplies abound. Besides, an increase in resistance against many different drugs among *Shigella* isolates has been observed in the last two decades.

Objective: To determine the pattern of antimicrobial sensitivity of shigella species to commonly used antibiotics.

Methods: The antibiotic susceptibility pattern of *Shigella* species isolated from stool specimens processed over five years at the bacteriology laboratory of the University of Gondar teaching hospital in northwest Ethiopia was investigated. Stool specimens were processed in accordance with the standard bacteriological methods and data were collected in the laboratory log book for bacterial culture, isolation and antimicrobial sensitivity.

Results: From the total of 2,891 stool specimens that were cultured, 214 yielded *Shigella* species. The overall sensitivity of *Shigella* species to the commonly used antibiotics in the area was 92.1% to gentamicin, 91.1% to ciprofloxacin, 47.2% to chloramphenicol, 26.6% to cotrimoxazole, 20.1% to ampicillin and 14% to tetracycline. About 46% of the isolates were found to be resistant to at least three commonly used drugs, while 1.4% were found to be resistant to all the commonly used drugs.

Conclusion: The results demonstrate the continued sensitivity of *Shigella* species to gentamicin and ciprofloxacin and their widespread resistance against tetracycline, ampicillin and cotrimoxazole. Based on our results, gentamicin and ciprofloxacin may be used rationally under strict regulation for the treatment of severe cases of shigellosis in case where other safer and better drugs are unavailable. [*Ethiop.J.Health Dev.* 2006;20(3):194-198]

Introduction

Shigellosis is a global human health problem. It is still an important public health problem, especially in developing countries, where there is substandard hygiene and unsafe water supplies (1,2). *Shigella* still accounts for a significant proportion of bacillary dysentery in many tropical and subtropical countries (3,4). It is the most prevalent etiologic agent in childhood diarrhoea in most countries (5,6). In Ethiopia, *Shigella dysenteriae* and *Shigella flexineri* have been identified as the species that account for about 80% of *Shigella* isolates (2).

The emergence of antimicrobial resistance to members of the Enterobacteriaceae family is posing serious problems in the treatment of outbreaks of infections. Since its first report in studies conducted in the 1950s, multiple-drug resistance transmitted by plasmids among *Shigella* species has been reported from many countries (6-8). Moreover, an increase in resistance against many different drugs has been observed in the last two decades. In one study, a significant decrease was observed in the susceptibility of the species to ampicillin and cotrimoxazole from 1988-89 to 1991-92 (9). In another report, it was shown that cotrimoxazole resistance of *Shigella* increased from 3% to 40% within ten years time (10). Another study showed that the percentage of resistant *Shigella* strains in Madrid (Spain) increased from 39.6% to 97.9% for ampicillin, tetracycline, and from 1.6% to 15.1% for chloramphenicol (11).

In Ethiopia, strains of *Shigella* that were resistant to many commonly used drugs have been reported in different parts of the country by several studies (12-14). In the aforementioned Ethiopian study reports, the strains were found to be most commonly resistant to tetracycline (>80%), ampicillin (>65%), and cotrimoxazole (>70%). Multiple drug resistance to ampicillin, chloramphenicol, tetracycline, and streptomycin was also very high in those studies. Belay and his colleagues have reported a strain that was resistant to eight drugs out of the nine antimicrobials they used (14).

Besides the temporal changes in the antibiogram of *Shigella* species, it is well known that antibiotic susceptibility patterns in *Shigella* may differ between geographical areas. Such differences are never stable and may change rapidly especially in places where antibiotics are used excessively (particularly in developing countries) (15). This warrants for frequent observation on the change in the pattern of antibiogram for this organism.

To our knowledge, no report exists regarding the antibiotic resistance pattern of *Shigella* species in Gondar region except one in the previous decade (16).This study was thus carried out to determine the pattern of

from 34.4% to 96.9% for cotrimoxazole, from 6.3% to 18.0% for

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antimicrobial sensitivity of *Shigella* species to the most commonly used antibiotics in Gondar, northwest Ethiopia.

Methods

This retrospective study was conducted in the bacteriology laboratory of the University of Gondar Hospital, Gondar, Northwest Ethiopia, between September 2001 and August 2005. The hospital is a tertiary level teaching and referral hospital currently rendering health services for over 4 million rural and urban inhabitants in northwest Ethiopia.

Stool specimens were collected from in- and out-patients during the five year period and were processed following the standard bacteriological methods (1). The stool specimens were inoculated on plates of MacConkey agar (DIFCO) and Salmonella-Shigella agar (DIFCO). The plates were incubated at 37ºC for 24 hours and suspected colonies were further examined by conventional biochemical tests. Antimicrobial sensitivity testing was carried out by the standardized agar disk diffusion techniques on Mueller Hinton agar (DIFCO) using the following antimicrobial agents: ampicillin (A), tetracycline (T), cotrimoxazole (SXT), gentamicin (CN), chloramphenicol (C), and ciprofloxacin (CIP) (17, 18). Turbidity of the broth was equilibrated to match with 0.5 McFarland standard.

The standard reference strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were tested as controls on the biochemical tests and agar plates including Mueller Hinton with antibiotic discs. Data on age, sex, source of specimens and culture results were collected from the bacteriology log book. In this study, multi-drug resistance was defined as simultaneous resistance to three or more antimicrobial agents. Statistical analysis was made using SPSS version 11.5 software. Ethical clearance was obtained from the Research and Publication office of the University of Gondar.

Results

A total of 2891 stool specimens were received from inpatients (n=2371 (82%)) and out-patients (n=520 (18%)) and processed in the bacteriology laboratory over the five years period. A total of 214 *Shigella* species were isolated from the stool specimens, while 42 other entries with incomplete data were excluded from the analysis. Specimens cultured from children (up to 15 years of age), adults aged between 16 to 45 years and those above 45

Table 3: **Comparison of resistance rate of *Shigella* species to commonly used antibiotics in different regions (Addis Ababa versus Gondar) and in different periods (1980, 1995, 2001-2005)**

Antibiotics	1980 (Addis Ababa) Ref. 20	1994-96 (Addis Ababa) Ref. 16	2001-2005 Gondar (Preset study
Ampicillin	61.2	90	79.9
Chloramphenicol	64.2	77.8	52.8
Cotrimoxazole	0	72	73.4
Tetracycline	39.5	91.2	86
Gentamicin	0	2	7.9
Ciprofloxacin	-	-	8.9

years accounted for 36.4%, 53.3%, and 10.3%, respectively. One hundred fifteen (53.5%) of the isolates were from male patients and the rest 99 (46.5%) were from females. The majority of the *Shigella* species (N=169, 75.2%) were isolated from specimens collected from patients at the outpatient department and the rest (N=53, 24.8%) were from stool specimens collected from in-patients. The distribution of the isolates in the different years was 45 (20.9%) in 2001, 23 (10.3%) in 2002, 34 (15.8%) in 2003, 85 (39.5%) in 2004, and 27 (14.6%) in 2005.

The i*n vitro* antimicrobial susceptibility pattern of *Shigella* isolates identified in the University of Gondar Hospital from September 2001-August 2005 is shown in Table 1. About 46% of the *Shigella* isolates were found to be resistant to at least three commonly used drugs, and 1.4% of the *Shigella* isolates were found to be resistant to all the commonly used drugs. Overall, many different patterns of resistance were observed. The predominant resistance patterns to triple antibiotics were those to A,T,Sxt (60.3%), A,T,C (46.3%), and A,C,Sxt (41.1%). The antibiogram resistance patterns for three up to six antibiotics are depicted in Table 2.

The highest prevalence of antibiotic resistance was documented to be against ampicillin 171 (79.9 %), tetracycline 184 (86 %), and cotrimoxazole 157 (73.4%). The least resistance of antibiotics was observed to ciprofloxacin 19(8.9%) and gentamicin 17(7.9%). Over the years from 2001 to 2005, there was no significant increase in resistance to the individual antibiotics tested.

Table 1: ***In vitro* antimicrobial susceptibility pattern of *Shigella* species isolate in University of Gondar Hospital from September 2001 to August 2005 (N=214)**

Antibiotic	Susceptible N (%)	Resistant N (%)
Ampicillin	43 (20.1)	171 (79.9)
Cotrimoxazole	57 (26.6)	157 (73.4)
Tetracycline	30 (14)	184 (86)
Chloramphenicol	101 (47.2)	113 (52.8)
Gentamicin	197 (92.1)	17 (7.9)
Ciprofloxacin	195 (91.1)	19 (8.9)

Table 2: **Multiple drug resistance patterns among *Shigella* species isolated in University of Gondar Hospital, north West Ethiopia, September 2001 to August 2005.**

Resistance types	Resistant isolates N (%)
R to 3 antibiotics	
A, T, C	99(46.3)
A, C, SXT	88 (41.1)
A, T, SXT	129 (60.3)
A, SXT, CIP	12 (5.6)
R to 4 antibiotics	
A, T, C, SXT	85 (39.7)
A, C, SXT, CIP	6 (2.8)
A, T, SXT,CIP	12 (5.6)
R to 5 antibiotics	
A, T, C, SXT, CN	9 (4.2)
A, C, SXT, CN, CIP	4 (1.9)
R to 6 antibiotics	
A, T, C, SXT, CN, CIP	3 (1.4)

Discussion

This retrospective study demonstrated a high level of antimicrobial resistance pattern in *Shigella* species isolated from stool samples over a five year period in Gondar, northwest Ethiopia. The fact that the majority of *Shigella* isolates are from the pediatric population is reported in the literature where 70% of all infections occur in children younger than 15 years (19). In the present work, children accounted for more than a third (36.4%) of all *Shigella* positive patients.

In line with our findings, a low rate of resistance to gentamicin and ciprofloxacin was observed in different studies (4, 14). For example, Moez and his colleagues observed a 3.1% resistance to ciprofloxacin (4). A recent prospective study conducted in southern Ethiopia showed a high sensitivity of *Shigella* isolates to gentamicin (14).

Resistance to cotrimoxazole, one of the drugs used in the treatment of shigellosis was first reported in Ethiopia in 1981 (20). Prior to this period, all strains isolated in Addis Ababa were found to be sensitive to cotrimoxazole and gentamicin (20). However, Belay and his colleagues (14) demonstrated a 55% resistance of *Shigella* strains to cotrimoxazole. Although Khan-Mohammed *et al* (5) showed a 2.7% resistance rate in Trinidad, our finding of a 73% resistance rate of the isolates to this antibiotic was in agreement with observations from Pakistan (3) and Iran (4) where a resistance rate of 70.4% and 87.8%, respectively, by *Shigella* species to cotrimoxazole was reported.

In northwest Ethiopia, the resistance of *Shigella* species to cotrimoxazole is high and has slightly increased in comparison to the previous report (16) (Table 3).

Unfortunately, the drug is still commonly prescribed and used by the community for diarrhoeal diseases, particularly in children, and on the bases of self prescription from private pharmacies (21-23). Therefore, the fact that the majority of *Shigella* isolates were resistant to cotrimoxazole is depressing since this drug is one of the drugs of choice in many countries (19) including Ethiopia.

Compared to a previous report from Gondar (16), a four fold increase in resistance to gentamicin was observed in the present study (from 2% to 7.9%) (Table 3). This increase might be because of the relatively increased use of gentamicin and related aminoglycosides (e.g., streptomycin) over the past five years (21-23). Since this antibiotic appears to be less commonly used than the orally administered antibiotics, the rate of resistance is not yet high in this study as well as in other recent studies from Bangladesh (24) and Sudan (25). Gentamicin and ciprofloxacin are the only two commonly available antibiotics to which the majority of *Shigella* strains in Gondar area were susceptible as seen from the present study. Although ciprofloxacin is contraindicated for those patients below 18 years of age, gentamicin can still be used in settings where safer drugs are not available.

Of the *Shigella* isolates, 90.8% were found to be resistant to one or more antimicrobial agent(s), and 87.8% were multi-drug resistant. The most common resistance was to tetracycline (73.5%) and cotrimoxazole (70.4%) as was observed by other authors (4, 14). A high rate of resistance of *Shigella* species was also observed for cotrimoxazole (87.75%) and ampicillin (55.5%) in Pakistan and Nigeria (3, 26). On the contrary, KhanMohammed and his coworkers have found a low level of resistance to the above antibiotics as only 8.1% of the isolates exhibited resistance to ampicillin, 2.7% to chloramphenicol, and 2.7% to cotrimoxazole (5).

In this study, multiple drug resistance to as many as five and six antibiotics was observed. Similar findings were seen in other studies from other localities in Ethiopia (1214, 20). S*higella* isolates from Sudanese patients with diarrhoea also showed high resistance rates against the commonly used antimicrobial agents: ampicillin, chloramphenicol, tetracycline, cotrimoxazole, and sulfonamide (25). Another study conducted in six countries in the East African region also showed resistance of *Shigella* isolates to the commonly used antibiotics except for nalidixic acid (27). A WHO scientific working group had reported a high prevalence of resistance to A, C, T and Sxt in developing countries because of the very high consumption of antibiotics from the open market in these countries (15). Similarly, this antibiogram was also common in our setting accounting for 40% of the isolates (Table 2). Yet, the current treatment of choice for shigellosis are still cotrimoxazole, ampicillin and ciprofloxacin (19,28).

Shigella are notorious for the rapid emergence and spread of multiple drug resistance among strains and resistance

patterns observed among strains of any one species in a single area may be numerous (16, 29) as also noted in our findings. The measures suggested to delay the development of resistance to cotrimoxazole in the days when it was a potent drug against the then prevalent strains hold true for fluoroquinolones and gentamicin today. Unfortunately, the orally administered fluoroquinolones are sold at private pharmacies without prescription in the region and the urban community has become well-acquainted with those antibiotics for the treatment of common infections. Unless the unrestricted use of these antibiotics is stopped in the area, the time that these antibiotics become ineffective in the treatment of severe shigellosis (such as cotrimoxazole and chloramphenicol) is not far.

The pitfalls in the study include the fact that we could not control the bacteriological methods and the data collection. There were only six antibiotic discs used for the sensitivity testing and antibiotic discs such as nalidixic acid, cephalosporins and norfloxacin were not included in the battery. Important data such as the nature of the diarrhoea (whether dysenteric, persistent or acute watery) could not be obtained. Such measures as incidence of shigellosis could not also be calculated from such a retrospective study.

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