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ANTIBIOTIC RESISTANCE AND SERO-GROUPS OF *SHIGELLA* AMONG PAEDIATRIC OUT-PATIENTS IN SOUTHWEST ETHIOPIA
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ABSTRACT

Objective: **To determine the prevalence of *Shigella* sero-groups and resistance pattern of isolates to commonly used antibiotics in Jimma.**
Design: **Cross-sectional survey.**
Setting: **The study was conducted in Jimma, southwest Ethiopia.**
***Subjects*: A total of 384 paediatric out-patients with diarrhoea aged 14 years and below were studied.**
Methods: **Stool specimens were collected from children presenting with diarrhoea using Cary-Blair transport medium and buffer treated swabs from Jimma hospital and Jimma health centre. Isolation, biochemical characterisation, sero-grouping and antibiotic sensitivity testing were performed according to standard methodology in the Microbiology laboratory of Jimma University.**
Results: **Out of the 77 *Shigella* strains isolated, sero-group A comprised 29.9%, B 40.3%, C 19.5% and D 10.4%. Among all *Shigella* sero-groups, highest resistance was encountered to tetracycline (63.6%), ampicillin (70.1%), cephalothin (57.1%), trimethoprim-sulphamethoxazole (32.5%) and chloramphenicol (40.3%) while least resistance was observed to gentamicin (1.3%), polymyxin B (3.9%) and nalidixic acid (6.5%).**
Conclusion: **Gentamicin, polymyxin B and nalidixic acid were found to be the drugs of choice for cases related with shigellosis.**

INTRODUCTION

In developing countries, diarrhoea is an important cause of morbidity and mortality, especially among children mainly due to poor socio-economic factors and sanitary conditions(1). Together with malaria, cholera and influenza, bacillary dysentery (shigellosis) has been one of the world's great scourges. Shigellosis is primarily a paediatric disease, with more than half of all infections occurring in children between six months to 10 years of age(2), although it can affect susceptible individuals at any age who are subject to poor sanitation(2). It is transmitted by the faecal-oral route and the rate of transmission is increased by the low inoculum of bacteria necessary to produce illness in man.

In many countries, a high incidence of antibiotic resistance has been observed in *Shigella*(3,4). Widespread out breaks of shigellosis due to multiple antibiotic resistant *Shigellae* has been documented in Central America(5), Asia(6) and Africa(7,8). Factors contributing to the emergence of drug resistance include use of sub-therapeutic doses of antibiotics as well as their use in animals and animal feeds(1,2).

In Ethiopia, a limited number of studies on the prevalence of shigella and associated drug resistance have been carried out mainly in Addis Ababa(9-12). Considering the magnitude of the problem, research on aetiology of shigella has been very limited in Ethiopia. The purpose of the present study was therefore to determine its prevalence and antibiotic resistance pattern in Jimma. It is hoped that it will serve as a base-line study for future *Shigella* studies in south western Ethiopia.

MATERIALS AND METHODS

The study was carried out in Jimma, a large town located 335 kms southwest of Addis Ababa which acts as a commercial and administrative centre of its region. It is overcrowded though some areas are sparsely populated but with poor sanitation.

A total of 384 diarrhoeal stool specimens from paediatric out-patients (age ≤14 years) were collected using sterile Cary-Blair transport medium and buffer treated swabs(13,14) from Jimma hospital (teaching and referral hospital) and Jimma health centre between March 2000 and July 2000. The specimens were analysed in the Microbiology laboratory of Jimma University (former Jimma Institute of Health Sciences). Each specimen was plated directly on Oxoid primary media (Oxoid, England): MacConkey agar and *Salmonella-Shigella* agar (SS). For enrichment, selenite enrichment broth was used. All were incubated at 37°C for 18-24 hours. Characteristics colonies were selected and picked for biochemical characterisation using standard methods(13,14).

Shigella isolates were sero-grouped by slide agglutination tests using Shigella polyvalent and group antisera (*Shigella* group A, B, C, D antisera) from Difco laboratories Inc, USA following standard procedures(13,14). For control purposes, a drop of saline was placed on another slide and bacterial cultures were emulsified without antiserum. *Shigella* strains whose sero-groups were known were used as positive controls.

The Bauer-Kirby(15) standard procedure was used for the following antibiotics (obtained from Difco, USA) with stated amounts and abbreviations on Muller-Hinton agar. Ampicillin (AM, 10 μg), carbenicillin (CB, 100 μg), cephalothin (CE, 30 μg), chloramphenicol (CL, 30 μg), gentamicin (GM, 10 μg), kanamycin (K, 30 μg), polymyxin B (PB, 300 units), tetracycline (TE, 30 μg), trimethoprim-sulphamethoxazole (SxT, 25 μg) and nalidixic acid (NA, 30 μg). Following a standard interpretative table(15), the inhibition diameters were measured to the nearest millimetre for interpretation as resistant, intermediate or susceptible. A standard reference strain of *E. coli* (ATCC 25922), which is sensitive to all these drugs was used for a quality control).

Only one strain in sero-group A and B each were found to be susceptible to all antibiotics tested while 97.4% of the isolates were found to be resistant to one or more drugs (Table 2). Gentamicin, polymyxin B and nalidixic acid were found to be the most effective antimicrobials, at least *in vitro,* whereas tetracyclines, ampicillin and cephalothin were the least effective for all serogroups.

A total of 24 distinct antibiograms were encountered in all *Shigella* strains and the patterns varied from resistance to a single antimicrobial agent to that of six (Table 3). Frequently encountered antibiograms were ampicillin,

Table 1

Antimicrobial susceptibility of Shigella isolated from paediatric out-patients

SSG	No. (%)	Number of strains susceptibility to* (%)									
		AM	CB	CE	CL	GM	K	PB	TE	SxT	NA
A	23 (29.9)	6 (26.1)	14 (60.9)	8 (34.8)	11 (47.8)	23 (100)	18 (78.3)	21 (91.3)	8 (34.8)	14 (60.9)	21 (91.3)
B	31 (40.3)	9 (29)	20 (64.5)	14 (45.2)	18 (58.1)	30 (96.8)	27 (87.1)	31 (100)	11 (35.5)	22 (71)	28 (90.3)
C	15 (19.5)	5 (33.3)	11 (73.3)	5 (33.3)	11 (73.3)	15 (100)	15 (100	14 (93.3)	7 (46.7)	10 (66.7)	15 (100)
D	8 (10.4)	3 (37.5)	5 (62.5)	6 (75)	6 (75)	8 (100)	7 (87.5)	8 (100)	2 (25)	6 (75)	8 (100)
All	77 (100)	23 (29.9)	50 (64.9)	33 (42.9)	46 (59.7)	76 (98.7)	67 (87.0)	74 (96.1)	28 (36.4)	52 (67.5)	72 (93.5)

SSG=*Shigella* serogroups identified; No. = Number of strains
* AM = Ampicillin, CB = Carbenicillin, CE = Cephalothin, CL = Chloramphenicol, GM = Gentamicin, K = Kanamycin, PB = Polymyxin B, TE = Tetracycline, SxT = Trimethoprim-Sulphamethoxazole, NA = Nalidixic acid

RESULTS

The frequency of occurrence of sero-groups A, B, C and D among the 77 *Shigella* isolates is shown in Table 1. The most frequently isolated sero-group was B while the least frequent was D.

Susceptibility of *Shigella* isolates to antimicrobials is shown in Table 1. The susceptibility of all strains in each serogroup to ampicillin, tetracyclines and cephalothin was below 47% (except for a 75% susceptibility of serogroup D to cephalothin), while less than 50% of serogroup A strains were susceptible to chloramphenicol. Sixty to seventy five per cent of the isolates were found to be susceptible to trimethoprim-sulphamethoxazole and carbenicillin in each sero-group, while more than 90% of the isolates were found to be susceptible to gentamicin, polymyxin B and nalidixic acid.

Table 2

Antimicrobial resistance of Shigella isolated from paediatric out-patients

SSG	NS tested	NSR to one or more drugs	%	NSR to two or more drugs	%
A	23	22	95.7	20	87
B	31	30	96.8	27	87.1
C	15	15	100	13	86.7
D	8	8	100	6	75
All	77	75	97.4	66	85.7

SSG = Shigella serogroups; NS = Number of strains; NSR = Number of strains resistant

Table 3

Resistance antibiograms of Shigella isolates

SSG	No.	Resistance antibiogram*	No
A	22	AM	2
		AM, TE	3
		AM, TE, CE	3
		AM, TE, CE, CL	3
		TE, CE, CB, CL	2
		CE, CB, CL, SxT, K	3
		AM, K, SxT, PB, NA	2
		AM, TE, CE, CL, CB, SxT	4
B	30	AM	2
		TE	1
		AM, TE	4
		AM, CE	4
		TE, CE	4
		AM, CE, CL	2
		TE, CE, NA	2
		AM, CE, CL, CB	2
		TE, CL, CB, SxT, NA	1
		AM, CE, TE, CL, CB, SxT	3
		AM, TE, CL, CB, SxT, GM	1
		AM, TE, CL, CB, SxT, K	4
C	15	AM	1
		CE	1
		AM, CE	1
		AM, TE, CE	4
		AM, CB, CL	3
		TE, CE, SxT	3
		CE, SxT, CB	1
		AM, TE, CL, PB	1
D	8	TE	1
		AM	1
		CE, SxT	1
		AM, TE, CB	3
		TE, AM, CL	1
		TE, CE, CL, SxT, K	1

SSG = Shigella serogroups; No.=Number of strains
*Abbreviations of antibiotics is as in materials and methods

tetracyclines, cephalothin, chloramphenicol and trimethoprim-sulphamethoxazole. No strain was resistant to all antibiotics tested but four strains of sero-group A and eight strains of serogroup B were found to be resistant to six antibiotics (Table 3).

DISCUSSION

The isolation of *Shigella* from 20.1% (77/384) of paediatric out-patients with diarrhoea in this study was higher than the 7.7% isolation rate from Djibouti(7), 11.6% from Manila, Philippines(6), and 7.1 to 11% from Addis Ababa, Ethiopia(9-11). This higher prevalence of *Shigella* in Jimma may reflect the poor sanitary condition of the town and the endemicity of shigellosis in the area. Kebede and Mirgissa(16) have previously pointed out at the unsatisfactory practice of good sanitation and personal hygiene of mothers who are responsible for food preparation and child rearing. The same was found to hold true in Jimma with such conditions being associated with a high incidence of shigellosis(2,17).

Among shigella isolates, serogroup B *(Sh. flexneri)* was the most dominant (40.3%) followed by serogroup A *(Sh. dysenteriae)* (29.9%) and serogroup C (*Sh. boydii*) (19.5%). The predominance of *Sh. flexneri* in developing countries is well known(2,3,13). As in other parts of Ethiopia prominance of *Sh. flexneri* was confirmed in this study(9-12). All four serogroups co-exist in different proportions as in other developing countries(2,3,6). In developed countries, *Sh. flexneri* has given way to *Sh. sonnei* as the dominant serogroup(2,13).

More than 53% of *Shigella* isolates in each serogroup were resistant to tetracycline, ampicillin, cephalothin and also 25 to 40% of the isolates were resistant to trimethoprim-sulphamethoxazole, chloramphenicol and carbenicillin. This is much higher than what has been reported in previous studies done in Addis Ababa, Ethiopia in the early 1980's(10,11,18), but comparable to the recent reports from Addis Ababa and northwest Ethiopia(9,12,19). The resistance to all other antibiotics in this study is also much greater than that reported in earlier reports in the 1980's. It could be concluded from this and previous studies in Ethiopia that relatively recent isolates of *Shigella* strains examined tend to be more resistant to commonly used antibiotics than earlier ones(9-12,18,19).

The most common resistance antibiograms among *Shigella* isolates were those combinations containing ampicillin, tetracycline, cephalothin and chloramphenicol. *Shigella* isolates resistant to multiple drugs have been reported by number of authors(2,3,5,9-12,20,21). As noted by Murray(3) and WHO(22), globally and in developing countries in particular, the most common pattern is also resistance to four or more antibiotics, involving in particular ampicillin, tetracycline, chloramphenicol, sulphonamides and streptomycin. Murray(3) also pointed out that resistance is encountered more with those antibiotics misused or used frequently for therapeutic and/or prophylactic purposes. The high frequency of multiple antibiotic resistant *Shigella* isolates observed in this study most probably reflects the ease of access and the extensive use of antibiotics in Jimma and probably across the entire country.

More than 96% of the *Shigella* isolates in this study were susceptible to gentamicin and polymyxin B while almost 94% of the strains were susceptible to nalidixic acid *in vitro*. However, the clinical effectiveness of these drugs in the face of resistance to first line antibiotics has not previously been appreciated, except for nalidixic acid(5,17,20). As nalidixic acid was found to be effective *in vitro* in this study, it could thus be recommended for therapy of shigellosis in this country.

This baseline study on genus *Shigella* has identified currently prevalent serogroups and their resistance to the first line antibiotics in Jimma. Further investigations are recommended in order to observe the change of serogroup prevalence with time, detect and monitor the drug susceptibility pattern, identify most prevalent serotypes in the region as well as in the country and enhance epidemiological study of Shigella like phage typing and plasmid analysis.

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SCIENTIFIC LETTER

Dear Sir,

FOAMY AND SEA-BLUE HISTIOCYTES IN A CHILD

The sea-blue histiocyte syndrome is a congenital, hereditary histiolipidosis due to an inborn enzymatic error. It is well known that sea-blue histiocytes are frequently associated with a variety of systemic, haematological, and neurological syndromes(1,2). Here, we report a child who had foamy and sea-blue histiocytes in liver and bone marrow specimens associated with pulmonary and cutaneous involvement.

A 5.5-year old boy was admitted with a two-week history of pain and swelling on abdomen. Firstly, he was taken to a physician about three years before admission to our hospital because of general body trauma, and hepatosplenomegaly was diagnosed. One week after the trauma he had had a rectal bleeding for one month, but no aetiologic cause was detected. The family history was non-contributory. On physical examination his vital signs were normal. The weight and height were 17 kg (25th percentile) and 103 cm (3-10th percentile) respectively. Non-pruritic eryhematous and maculopapular rash was noted on the abdomen and back region. The enlarged liver was palpable 10 cm below the right costal margin and the spleen was enlarged to palpate 8 cm below the left costal margin. Other systemic findings were normal. On laboratory investigation routine urine and blood analyses including peripheral blood smear and sedimentation rate were normal. Serum electrolytes, renal and liver function tests were also normal. Serum total bilirubin was 3.9 mg/dl, conjugated bilirubin 1.8 mg/dl, aspartate aminotransferase 45 U/L, alanine aminotransferase 20 U/L, alkaline phosphatase 176 U/L, total protein 7.3 g/dl, albumin 3.9 g/dl, protrombin time 15 second and activated partial thromboplastin time 40 second. Hepatitis B surface antigen was negative. Serum immunoglobulin A, G and M levels were also normal. Thorax radiography and computerised tomography of the thorax revealed the presence of bilateral diffuse reticulonodular pulmonary infiltration. Computerised tomography of the abdomen showed hepatomegaly, splenomegaly associated with widespread nodular formation on the spleen and para-aortic lymphadenopathies. The barium oesophagogram was normal. Occult blood test on the stool was negative. Agglutination tests for salmonellosis and brucellosis were negative. Electrocardiographic and echocardiographic examination were normal. Histopathological examination of the skin showed widespread mononuclear cell infiltration on the dermal areas. Liver biopsy and bone marrow aspiration specimens showed the presence of foamy and sea-blue histiocytes. These histiocytes were closely packed with granules, dyed sea-blue with May-Giemsa staining.

The actual origin of sea-blue histiocytes is unknown. Though the precise biochemical defect can not always be identified, it seems reasonable to suggest that in most instances, a structurally defective or partially or completely absent enzyme leads to an accumulation of its natural substrate within the cells(3). Numerous miscellaneous diseases including Niemann-Pick disease associated with sea-blue histiocytes have been reported in the literature(1,4,5). Although, we were not able to do enzyme assays for the diagnosis of Niemann-Pick disease, the clinical and laboratory findings of our patient were most likely compatible with non-neuropathic Niemann-Pick disease. On account of this case we would like to emphasize that children with hepatosplenomegaly should carefully be examined for foamy and sea-blue histiocytes to avoid late diagnosis as in our case.

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