ORIGINAL ARTICLE

Salmonella Serogroups and their Antibiotic Resistance Patterns Isolated From Diarrhoeal Stools of Pediatric Out-Patients in Jimma Hospital and Jimma Health Center, South West Ethiopia

Abebe Mache, MSc*

ABSTRACT

BACKGROUND: *Salmonellosis is a major health problem, especially among children in developing countries. Moreover, Salmonellae are becoming resistant to commonly used antimicrobials in most parts of the world. Investigation on the Salmonella has been very limited in Ethiopia and such study lacks in Jimma. The aim of this study was to determine the prevalent salmonella serogroups and resistance pattern of the isolates to commonly used antibiotics in the study area.*

METHODS: *The study was conducted from March to July 2000. Diarrhoeal stool specimens were collected from 384 pediatric diarrhoeal out-patients (age ≤ 14 years) using transport medium from Jimma hospital and Jimma health centre. Isolation and characterization were performed according to standard methodology.*

RESULTS: *Fifty nine Salmonella strains were isolated, of which serogroup A comprised 8.5%, B 28.8%, C 22%, D 13.6%, E 5.1% and S.typhi 22%. Among the isolates, 59.3% were resistant to tetracycline and ampicillin, 47.5% to cephalothin, 40.7% to trimethoprim-sulfamethoxazole, 35.6% to chloramphenicol, and less than 25.4% were resistant to other drugs. Among S.typhi isolates, 30.8% were resistant to chloramphenicol and this shows the emergence of chloramphenicol resistant S.typhi strains in Jimma.*

CONCLUSION: *Gentamicin, polymyxin B and nalidixic acid were found to be active against isolates of salmonella species including* S. typhi.

KEY WORDS: *Diarrhoea, Salmonella, Serogroup, Antibiotic resistance, Pediatrics, out-patient.*

*Department of Medical Microbiology and Parasitology, Jimma University, P.O. Box 482, Jimma.

INTRODUCTION

Salmonellosis is usually food-borne and generally results from the consumption of contaminated foods that have been mishandled. However, person-to-person spread may occur, especially in pediatric wards, nurseries and nursing homes (1). It is a major health problem, especially in developing countries where conditions still favor endemic disease and the incidence of the disease is greatest in children (1,2).

The real incidence of salmonellosis among children in many countries is not well known and comparison of the number of isolations may be misleading owing to variations in population characteristics, under-reporting and differences in epidemiological and laboratory technigues (1,3). For example, studies in some developing countries showed that *Salmonellae* are isolated from 1 to 8% (3) of children with diarrhoea and in some urban areas of developing countries, where industrially processed food is commonly consumed, cases and outbreaks of *Salmonella* infection in man, especially in children, are found to be even more common than in rural areas (1,4).

In many countries, high incidence of antibiotic resistance has been observed in *Salmonella* (4,5). Widespread out breaks of salmonellosis due to multiple antibiotic resistant *Salmonellae* has been documented in Central America (6), Asia (7) and Africa (8, 9). Factors contributing to the emergence of drug resistance include non-administered and sub-therapeutic use of antibiotics as well as the use of antibiotics in animals and animal feeds (2,3).

In Ethiopia, a limited number of studies of the genus *Salmonella* concerning drug resistance and /or prevalent serogroups have been made mainly in Addis Ababa (10-13). Considering the magnitude of the problem, investigation of the aetiologic agent has been very limited in Ethiopia and no such study has been recorded in Jimma. The purpose of the present study was to determine the prevalent serogroups and present resistance pattern of the isolates to commonly used antibiotics in Jimma. It is hoped that it will serve as a base-line study for future *Salmonella* studies in Southwest Ethiopia.

MATERIALS AND METHODS

Sample size determination: As there was no reliable estimate of Salmonella infection in the study community, a 50% infection rate, which leads to the highest possible sample size was used as recommended by Daniel (14) and Parker (15). The estimate was desired to be with 5% margin of error and 95% confidence interval. The study was a cross-sectional and until the required sample size was attained, all children with diarrhoeal complaints visiting a hospital and the health center within the specified time were included in the study.

Collection of stool specimens: A total of 384 diarrhoeal stool specimens from pediatric out-patients (age≤14 years) were collected using sterile Cary-Blair transport medium and buffer treated, heavily charged swabs (16,17) from Jimma hospital (teaching and referral hospital) and Jimma health centre between March and July 2000. The study sites were selected based on the proximity to the microbiology laboratory of Jimma University and since such study is not conducted on Salmonella in Jimma, which is the largest town in the South Western Ethiopia with possibly higher degree of antibiotic usage than the rural vicinity. The specimens were analysed in the Microbiology laboratory of Jimma University.

Isolation and biochemical characterization: Each specimen was plated within 2-4 hours of collection directly on Oxoid primary media (Oxoid, England): MacConkey agar and *Salmonella-Shigella* agar (SS). For enrichment, Selenite F enrichment broth, which inhibits coliform bacilli and selective for *Salmonellae* was used (16, 17). All were incubated at 37°C for 18-24 hours. Characteristic colonies were selected and picked for biochemical characterization using Kligler iron agar, motility, indole, urea agar, mannitol and glucose broth with Durham tubes following standard methods (16,17).

Serogrouping: Salmonella isolates were serogrouped by slide agglutination tests using *Salmonella* polyvalent and group antisera (*Salmonella* group A, B, C, D, *S.typhi* and E antisera) all from Difco laboratories Inc, USA) following standard procedures (16,17). For control purposes, a drop of saline was placed on another slide and bacterial cultures were emulsified without antiserum. *Salmonella* strains whose serogroups were known were used as positive controls.

Antibiotic sensitivity testing: The Bauer-Kirby (18) standard procedure was used for the following antibiotics (all obtained from Difco, USA) with stated amounts and abbreviations on Muller-Hinton agar. Ampicillin (10 µg), carbenicillin (100 µg), cephalothin (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), polymyxin B (300 units), tetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg) and Nalidixic acid (30 µg). Following a standard interpretative table (18), the inhibition diameters were measured to a nearest millimetre for interpretation as resistant, intermediate or susceptible. Since the readings of "intermidiate" were very few, they were, for practical purposes, considered susceptible. A standard reference strain of *E.coli* (ATCC 25922), which was sensitive to all these drugs was used for a quality control. Descriptive statistics was used for interpretation of the data.

RESULTS

Among 59 *Salmonella* isolates, the frequency of occurrence of serogroups A, B, C, D (apart from *S.typhi)*, *S.typhi* and E is shown in Table 1. The most frequently isolated serogroup was B while the least frequent was E. S.typhi accounted for 22% of the isolates.

Susceptibility of *Salmonella* isolates to antimicrobials is shown in Table 1. Susceptibility of all serogroups to polymyxin B and gentamicin was found to be 100% except one strain in serogroup D and B respectively for each, while more than 91% of the isolates were suseptible to nalidixic acid. The susceptibility of all strains in each serogroup to ampicillin and tetracycline was below 48%. Between 52.5% to 74.6% of the isolates were sensitive to the other antibiotics.

Among the isolates, only 33.3% of serogroup E, 37.5% of serogroup D, 38.53% of serogroup C, 40% of serogroup A, 41.2% of serogroup B and 46.2% of *S. typhi* were susceptible to ampicillin. Susceptibility to tetracyclines varied from 20% to 47.1%, while less than 50% of serogroup C isolates were susceptible to cephalothin. About 59% of the total isolates were suceptible to carbenicillin, while 69.2% of *S. typhi* isolates were susceptible to chlaramphnicol. Two strains in serogroup B and one strain in serogroup D and *S. typhi* each were found to be susceptible to all antibiotics tested while 93.2% 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,* where as tetracyclines, ampicillin and cephalothin were the least effective for all serogroups.

A total of 31 distinct antibiograms

were encountered in all *Salmonella* strains and the patterns varied from resistance to a single antimicrobial agent to that of six (Table 2). Frequently encountered antibiograms were ampicillin, tetracyclines, cephalothin, carbenicillin, chloramphenicol and trimethoprim-sulphamethoxazole. No strain was resistant to all antibiotics tested but one strain in serogroup A was found to be resistant to six antibiotics (Table 2).

Table 1. Antimicrobialsusceptibility of Salmonella isolated from pediatric diarrhoeal out-patients in Jimma Hospital and Jimma Health Center, Jimma, March-July 2000.

SSI	No %	Number of strains susceptibility to* %									
		AM	CB	CE	CL	GM	K	PB	TE	SxT	NA
A	5 (8.5)	2 (40)	3 (60)	2 (40)	3 (60)	5 (100)	3 (60.0)	5 (100)	1 (20)	3 (60)	4 (80)
B	17 (28.,8)	7 (41.2)	10 (58.8)	9 (53)	11 (64.7)	16 (94.1)	14 82.4	17 (100)	8 (47.1)	10 (58.8)	15 (88.2)
C	13 (22)	5 (38.5)	7 (53.9)	6 (46.2)	7 (53.9)	13 (100)	9 (69.2)	13 (100)	5 (38.5)	9 (69.2)	12 92.3
D	8 (13.6)	3 (37.5)	5 (62.5)	5 (62.5)	6 (75)	8 (100)	6 (75)	7 (87.5)	3 (37.5)	4 (50)	8 (100)
ST	13 (22)	6 (46.2)	8 (61.5)	7 (53.9)	9 (69.2)	13 (100)	10 (77)	13 (100)	6 (46.2)	8 (61.5)	12 (92.3)
E	3 (5.1)	1 (33.3)	2 (66.7)	2 (66.7)	2 (66.7)	3 (100)	2 (66.7)	3 (100)	1 (33.3)	1 (33.3)	3 (100)
All	59 (100)	24 (40.7)	35 (59.3)	31 (52.5)	38 (64.4)	58 (98.3)	44 (74.6)	58 (98.3)	24 (40.7)	35 (59.3)	54 (91.5)

SSI= Salmonella sero-groups identified; No.- number of strains; St= Salmonella typhi
*Ampiillin (AM); Gentamicin (GM); Carbenicillin (CB); Kanamycin (k); Cephalothin (CE);

Table 2. Single and multiple antimicrobial resistance of Salmonella isolated from pediatric diarrhoeal out-patients in Jimma Hospital and Jimma Health center, March-July 2000.

SSG	NST	NSROMD	NSRTMD	Resistance antibiogram*	No
A	5	4	4	TE	1
				AM,TE,CE	1
				CE, CL, K, SxT	1
				TE, AM, CE, CB, K	1
				TE, AM, CL, SxT, NA, CB	1
B	17	15	15	AM, TE	2
				CE, CL, TE	4
				AM, CB, SxT	3
				AM, CL, K, NA	1
				TE, CE, CB, SxT	1
				AM, TE, SxT, K, NA	1
				AM, TE, CE, CB, SxT	1
				AM, CE, CL, CB, GM	1
				AM, CE, CB, SxT, K	1
C	13	12	12	TE	1
				AM, TE	1
				AM, TE, CE	1
				CB, CE, CL	1
				TE, CE, SxT	3
				AM, CB, CL, K	3
				AM, K, SxT, NA	1
				AM, TE, CE, CB, CL	2
D	8	7	7	AM, TE	1
				TE, SxT	1
				AM, TE, SxT	1
				AM, CL, K, PB	1
				TE, SxT, CB, CE	1
				AM, CB, CE, CE	1
				AM, CB, CE, CL, K	1
				AM, TE, SxT, CB, CE	1
St	13	12	11	AM	1
				AM, TE	1
				AM, K, NA	1
				AM, TE, CE	1
				AM, SxT, K	2
				TE, CE, CB	1
				TE, CE, CB, CL	2
				TE, CE, SxT, CB	1
				AM, TE, SxT, CL	1
				CE, SxT, CB, CL	1
E	3	3	2	AM	1
				TE, SxT, CB, CE	1
				AM, TE, SxT, CL, K	1
	59	55	51		55

SSG= Salmonella serogroups, NST= Number of strains tested; NSROMD= Number of strains resistant to one or more drugs NSRTMD= Number of Strains resistant to two or more drugs; St= Salmonella typhi
*Abbreviation of antibiotics is as in materials and methods.

DISCUSSION

Isolation of 15.4% (59/384) *Salmonella* from pediatric diarrhoeal out-patients in this study was greater than the 2.9% isolation reported from Djibouti (8), 9.2% from Manila, Philippines (7), 3.3% from Lagos, Nigeria (9), and 4.5% to 10.9% from Addis Ababa, Ethiopia (10-13). This increased prevalence of *Salmonella* in Jimma may imply the sanitary condition of the town and the endemicity of salmonellosis in the area. Kebede and Mirgissa (19) have pointed out that people's practice of good sanitation is far from satisfactory and the personal hygiene status of the house mothers who are responsible for food preparation and child rearing was found to be poor in Jimma. Such conditions have been found to be significantly associated with incidence of salmonellosis (1,2).

Of all *Salmonella* isolates in this study, 78% (46\59) belong to non-typhi serogroups (serogroups A, B, C, D and E), which reflects the acute gastroenteritis nature of the majority of *Salmonella* related diarrhoea. The 22% (13\59) isolation of *S. typhi* in this study probably represents the post-systemic intestinal phase with diarrhoea since *S. typhi* primarily causes a systemic illness and the stool culture becomes positive for *S. typhi* after second and third week of the illness. This is almost similar to the results of Mogessie (13) and Mache *et al* (10) in Addis Ababa, Ethiopia. Messele and Alebachew in 1981 (11) and Afeworki in 1985 (12) also isolated different *Salmonella* serogroups from stool and blood specimens in Addis Ababa, Ethiopia, however, in their studies *S. typhi* was the dominant isolate, because most of their samples were from blood specimens.

The above stated frequency of isolation of *Salmonella* in this study may not necessarily indicate the frequency of occurrence or distribution of *Salmonella* serogroups among pediatric diarrhoeal out-patients in Jimma. Because unless their illness persists for several days, not many patients in Jimma seek medical attention for possible salmonellosis (personal communication with a pediatrician in Jimma Hospital), mainly due to lack of easy access to hospitals and health centers, and also because of the usually self-limiting nature of such diarrhoea, except causes related with *S.typhi.* This idea was supported by other researchers in Ethiopia (11, 12), as well as in the other parts of the world (1-3). For example, in the USA the number of reported cases of *Salmonella* infections is estimated to be only about 1-10% of the number of cases (1, 2).

More than 52% of *Salmonella* isolates in each serogroup were resistant to tetracycline and ampicillin and also 25 to 45% of the isolates were resistant to trimethoprim-sulfamethoxazole, cephalothin, chloramphenicol, and carbenicillin. This is much higher than previous studies reported from Addis Ababa, Ethiopia in the early 1980's (11-13), but comparable to the recent reports from Addis Ababa and North West Ethiopia in the late 1990's (10,20). The resistance to all other antibiotics in this study is also much greater than that reported by above authors in the 1980's. It could be concluded from this and previous studies in Ethiopia that relatively recent isolates of *Salmonella* strains examined tend to be more resistant than earlier ones to the first line antibiotics as noted by different authors (10-13, 20).

Moreover, 30.8% of the *S. typhi* isolates were resistant to chloramphenicol in this study while 100% of the *S. typhi* isolates were susceptible to this antibiotic in the early 1980s according to the findings of Messele *et al.* (11), Mogessie (13) and Afeworki (12). *S. typhi* resistant to Chloramphenicol is well documented in studies elsewhere (6, 21, 22), but this study

demonstrates the emergence of a significant level of chloramphenicol resistant *S. typhi* in the last decade in Ethiopia, especially in Jimma.

The most common resistance antibiograms among *Salmonella* isolates in this study were those combinations containing ampicillin, tetracycline, cephalothin and chloramphenicol. *Salmonella* isolates resistant to multiple drugs have been reported by number of authors (2,4,6,10-13,20). As noted by Murray (4) and WHO (23), globally and in developing countries in particular, the most common pattern is also resistance to 4 or more antibiotics, involving in particular ampicillin, tetracycline, chloramphenicol, sulfonamides and streptomycin. The high frequency of multiple antibiotic resistant *Salmonella* 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 as pointed out by Murray (4) and WHO (23).

More than 98% of the *Salmonella* isolates in this study were susceptible to gentamicin and polymyxin B while almost 92% of the strains were susceptible to nalidixic acid. According to in *vitro* susceptibility, these antibiotics were found to be the drugs of choice for cases related with salmonellosis including *S.typhi*. However, it is important to realize that in *vitro* inhibition or resistance can not be necessarily taken as sufficient evidence of a drug's effectiveness or lack of effectiveness in *vivo*.

To decrease the incidence of salmonellosis, public health measures such as improving personal and food hygiene and intensive health education has to be taken. In addition to this, developing well facilitated laboratory in this hospital and health center will help to prevent and control diarrhoeal diseases including salmonellosis and development of resistant strains against commonly used antibiotics. Such laboratory will help for prompt and definite identification of the etiologic agents and for periodically monitoring drug resistance for judicious drug prescription and therapeutic efficiency (4).

This base-line study on genus *Salmonella* has identified currently prevalent serogroups and their resistance to the first line antibiotics in Jimma. Further investigations are recommended in order to: (a) observe the change of serogroup prevalence with time (b) detect and monitor the drug susceptibility pattern and for recommending alternative therapy
(c) identify prevalent serotypes in the region as well as in the country (d) enhance epidemiological study of *Salmonella* like phage typing and plasmid analysis.

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