

Full Length Research Paper

Antibacterial susceptibility and Drug resistant pattern of Salmonella sero-groups from Pediatric out-patients at two Health centers in Nasarawa State, Nigeria

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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 Nasarawa State and such study lacks in Lafia The aim of this study was to determine the prevalence of salmonella sero-groups and resistance pattern of the isolates to commonly used antibiotics in Nasarawa State. The study was conducted from Jan to Feb 2013. Diarrhoeal stool specimens were collected from 384 pediatric diarrhoeal out-patients (age < 14 years) using transport medium from Dalhatu Araf Specialist Hospital and Federal Medical Center Keffi. Isolation and characterization were performed according to standard methodology. 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 Lafia. 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.

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 techniques (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 outbreaks 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 Nasarawa State, a limited number of studies of the genus Salmonella concerning drug resistance and /or prevalent serogroups have been made mainly in Nigeria (10-13). Considering the magnitude of the problem, investigation of the aetiologic agent has been very limited in Nasarawa State and no such study has been recorded in Lafia and Keffi. The purpose of the present study was to determine the

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prevalent serogroups and present resistance pattern of the isolates to commonly used antibiotics in Lafia and Keffi. It is hoped that it will serve as a base-line study for future *Salmonella* studies in Nasarawa State.

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 Dalhatu Araf Specialist Hospital and Federal Medical centre Keffi between January and Feb 2013. The study sites were selected based on the proximity to the Microbiology laboratory of Innovative Research Biotechnology Lab, Keffi.

Since such study is not conducted on *Salmonella* in Lafia and Keffi, which are the largest towns in the Nasarawa State with possibly higher degree of antibiotic usage than the rural vicinity. The specimens were analysed in the Microbiology laboratory of Innovative Research Biotechnology Lab, Keffi.

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 "intermediate" 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 susceptible 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 susceptible to carbenicillin, while 69.2% of *S. typhi* isolates were susceptible to chloramphenicol. 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

Table 1. Antimicrobial susceptibility of *Salmonella* isolated from pediatric diarrhoeal out-patients Dalhatu Araf Specialist Hospital and Federal Medical centre Keffi from Jan-Feb 2013.

SSI	No	%	Number of strains susceptibility to* %									
			AM	CB	CE	CL	GM	K	PB	TE	SxT	NA
A	5		2	3	2	3	5	3	5	1	3	4
	(8.5)		(40)	(60)	(40)	(60)	(100)	(60.0)	(100)	(20)	(60)	(80)
B	17		7	10	9	11	16	14	17	8	10	15
	(28.8)		(41.2)	(58.8)	(53)	(64.7)	(94.1)	82.4	(100)	(47.1)	(58.8)	(88.2)
C	13		5	7	6	7	13	9	13	5	9	12
	(22)		(38.5)	(53.9)	(46.2)	(53.9)	(100)	(69.2)	(100)	(38.5)	(69.2)	92.3
D	8		3	5	5	6	8	6	7	3	4	8
	(13.6)		(37.5)	(62.5)	(62.5)	(75)	(100)	(75)	(87.5)	(37.5)	(50)	(100)
ST	13		6	8	7	9	13	10	13	6	8	12
	(22)		(46.2)	(61.5)	(53.9)	(69.2)	(100)	(77)	(100)	(46.2)	(61.5)	(92.3)
E	3		1	2	2	2	3	2	3	1	1	3
	(5.1)		(33.3)	(66.7)	(66.7)	(66.7)	(100)	(66.7)	(100)	(33.3)	(33.3)	(100)
All	59		24	35	31	38	58	44	58	24	35	54
	(100)		(40.7)	(59.3)	(52.5)	(64.4)	(98.3)	(74.6)	(98.3)	(40.7)	(59.3)	(91.5)

SSI= *Salmonella* sero-groups identified; No.- number of strains; St= *Salmonella typhi*; *Ampicillin (AM); Gentamicin (GM); Carbenicillin (CB); Kanamycin (K); Cephalothin (CE); Polymyxin B (PB); Chloramphenicol (CL); Tetracycline (TE) Trimethoprim-Sulfamethoxazole (SxT); Nalidixic acid (NA).

found to be the most effective antimicrobials, at least *in vitro*, where astetracyclines, 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.

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 Lafia may imply the sanitary condition of the town and the endemicity of salmonellosis in the area. KofarKaura and Bukansidi (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 bearing was found to be in poor sanitary condition in Lafia. 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-typhiserogroups (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.

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 Dalhatu Araf Specialist Hospital, Lafia. Because unless their illness persists for several days, not many patients in Lafia seek medical attention for possible salmonellosis (personal communication with a pediatrician in Dalhatu Araf Specialist Hospital, Lafia), 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*), 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

Table 2. Single and multiple antimicrobial resistances of *Salmonella* isolated from pediatric diarrhoeal out-patients in Dalhatu Araf Specialist Hospital and Federal Medical centre Keffi from Jan-Feb 2013.

SSG	NST	NSROMD	NSRTMD	Resistance antibiogram ⁸	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

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 Lagos, Nigeria in the early 2007'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 Jameet al. (11),Ikeja (13) and Ibadan (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 Lafia.

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 Lafia 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 cannot 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 Lafia. 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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