Prevalence of Multi-Drug Resistant *Salmonella typhi* among Clinically Diagnosed Typhoid Fever Patients in Lagos, Nigeria

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A total of 635 clinically diagnosed typhoid fever patients were bled from three different health institutions in the metropolis of Lagos, Nigeria over a period of 15 months, May 1997 to July 1998.

Out of the total blood cultured, 101 (15.9%) isolates of *Salmonella* species were isolated of which 68 (67.3%) were *S. typhi*, 17 (16.8%) and 16 (15.8%) were *S. paratyphi A* and *S. arizonae* respectively. The overall isolation rate of *S. typhi* among patients is 10.7%, with most isolates 45.9% found among the severely-ill young adults, age group 16–30 years. All isolates were subjected to anti-microbial susceptibility testing using 12 different antibiotics: chloramphenicol, ampicillin, cotrimoxazole, gentamicin, colistin sulfate, nalidixic acid, nitrofurantoin, cefotaxime, tetracycline, streptomycin, ofloxacin and ciprofloxacin. All the *S. typhi* and *S. paratyphi* A isolates showed resistance to two or more of the 10 of 12 antibiotics tested particularly the 3-first-line antibiotics commonly used (chloramphenicol, ampicillin and cotrimoxazole) in the treatment of typhoid fever in Nigeria. No isolate showed resistance to ofloxacin and ciprofloxacin, however, nalidixic acid and gentamicin showed a moderate and appreciable inhibition to most of our isolates.

Introduction

Typhoid fever which is a common disease in Nigeria does not always present a distinct clinical picture, and other bacterial, viral and even protozoan infections may mimic its presentation (Levine *et al.*, 1978; Grange,1994). The febrile disease is among the major widely spread diseases affecting both young children and young adults in their productive years (Talabi, 1994). In 1992 alone, there were more than 19,000 documented typhoid cases and several lives were claimed (Anon,1993). The treatment of typhoid fever in Nigeria has been based mainly on antibiotics mostly used indiscriminately without prescription.

However in recent years, increasing resistance of *Salmonella typhi* to commonly used antibiotics has become a matter of concern. Strains of *S. typhi* resistant to the orally administered antibiotics such as chloramphenicol, ampicillin and cotrimoxazole have emerged as a major public health problem in Nigeria because of their abuse. In other parts of the globe, drug resistance in *S. typhi* has been known for the past 48 years (Mandal, 1994). Multi-drug resistant (MDR) *S. typhi* strains have become widely prevalent in India, Pakistan and China and have spread to the middle East and South of Africa (Mandal, 1994). Also multi-drug resistant typhoid fever is being encountered increasingly in Britain (Threlfall *et al.*, 1991), Egypt (Mourad *et al.*, 1993), Kuwait (Panigrahi *et al.*, 1996) and in the United States of America (Mistra *et al.*, 1997).

Materials and Methods

Study population and Centres

Over a period of 15 months (May 1997 to July 1998), blood samples were collected from 635 patients presented with typhoid fever and attending any of: Central Public Health Laboratory Services, Yaba, Lagos; Central Bank of Nigeria Health Clinics, Satellite Town Lagos, and Mainland Infectious Diseases Hospitals, Lagos. All the subjects were defined by the clinicians as having preaxial of 38 °C for up to 5 days with one or more of the following symptoms headache, vomiting, constipation, diarrhoea, abdominal pains cough, psychosis, loss of appetite, malaise and splemomegaly. The concepts of the study were explained to all the subjects, after which they were bled. Important bio-data such as age, sex, history of vaccination, and antimicrobial therapy were noted appropriately. All the patients recruited for this study cut across the low, middle and high socioeconomic class of the economy.

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Sample collection and bacteriology

In each case, 3 ml of blood were collected and inoculated directly into culture bottles containing Brain Heart Infusion broth (Oxoid, England). The culture bottles were incubated aerobically at 37 °C for 18–24 h. Subcultures were done onto plates of blood agar, *Salmonella-Shigella* agar and desoxycholate citrate agar and plates further incubated at 37 °C for 18–24 h. In negative cases subcultures were repeated from the infusion broth daily for 7 consecutive days before discarded. Isolates were identified by slide agglutination reactions using monospecific Somatic 'O' and Flagella 'H' specific phase antisera (Wellcome Diagnostics, London, UK), and combination of colonial and morphological characterisation on solid media together with biochemical reactions was as described by Cowan (1974).

Antimicrobial susceptibility testing

In vitro antimicrobial susceptibility testing was performed on all the isolates. Disc diffusion technique as described by Bauer *et al.* (1966) was adopted. In brief, the inoculum size was prepared by suspending five colonies of each pure bacterial culture into a sterile bijou bottle containing 5 ml of Mueller-Hinton broth (Oxoid, England). These inoculated media were incubated overnight at 37 °C. After dilution of the culture to about 10^{-4}, a sterile cotton swab was dipped into the adjusted inoculum and used to inoculate 20 ml of Mueller-Hinton agar (Oxoid, England) in a plate (Sterilin, UK) with the following antibiotics (Oxoid, England) per plate: ampicillin (25 μg), cotrimoxazole (25 μg), colistin-sulfate (10 μg), nalidixic acid (30 μg), nitrofurantoin (200 μg), cefotaxime (30 μg), tetracycline (50 μg), streptomycin (25 μg), chloramphenicol (30 μg), gentamicin (10 μg), ciprofloxacin (20 μg), and ofloxacin (20 μg). Plates with antibiotics discs were incubated for 24 h and zones of inhibition were measured in millimetre.

Results

Out of 635 subjects screened, 101 (15.9%) had bacteraemia and yielded isolates of *Salmonella* sp. out of which 68 (67.3%) were *S. typhi*, 17 (16.8%) were *S. paratyphi* while the remaining 16 (15.8%) were *S. arizonae*. Antibiotic sensitivity testing showed a very high percentage of resistance to the commonly used antibiotics: ampicillin, chloramphericol, cotrimoxazole and others (Table I). Moreover, all the 68 isolates of *S. typhi* and 17 of *S.*

Table I. Antimicrobial drugs resistance patterns among the *Salmonella* isolates.

Antibiotics Used	*Salmonella* isolates					
	S. typhi n = 68		*S. paratyphi* n = 17		*S. arizonae* n = 16	
	S (5)	R (%)	S (%)	R (%)	S (%)	R (%)
Amp	6 (8.8)	62 (91.2)	2 (11.8)	15 (88.2)	1 (6.3)	15 (93.8)
Cot	26 (38.2)	42 (61.8)	6 (35.3)	11 (64.7)	7 (43.8)	9 (56.3)
Chl	18 (26.5)	50 (73.5)	5 (29.4)	12 (70.6)	2 (12.5)	14 (87.5)
Gen	48 (70.6)	20 (29.4)	12 (70.6)	5 (29.4)	12 (75.0)	4 (25.0)
Nit	29 (13.2)	39 (57.4)	8 (47.1)	9 (52.9)	11 (68.8)	5 (31.3)
Tet	24 (35.3)	44 (64.7)	6 (35.3)	11 (64.7)	5 (29.7)	11 (68.8)
Str	46 (70.6)	22 (32.4)	11 (64.7)	6 (35.3)	10 (62.5)	6 (37.5)
Cef	33 (48.5)	35 (51.5)	11 (64.7)	6 (35.3)	11 (68.8)	5 (31.3)
Nal	59 (86.8)	9 (13.2)	14 (82.4)	3 (17.7)	14 (87.5)	2 (12.5)
Cip	68 (100.0)	0 (0.0)	17 (100.0)	0 (0.0)	16 (100.0)	0 (0.0)
Ofx	68 (100.0)	0 (0.0)	17 (100.0)	0 (0.0)	16 (100.0)	0 (0.0)
Col	38 (55.9)	30 (44.1)	10 (58.8)	7 (41.2)	10 (62.5)	6 (37.5)

Amp = Ampicillin
Cot = Cotrimoxazole
Chl = Chloramphenicol
Gen = Gentamicin
Cip = Ciprofloxacin
Str = Streptomycin
Tet = Tetracycline
Nal = Nalidixic acid
Nit = Nitrofurantoin
Col = Colistin Sulfate
Cef = Cefotaxime
Ofx = Ofloxacin
n = number of isolates
S = sensitive
R = resistant

Table II. List of antibiotics to which *Salmonella* isolates developed resistance.

s/No	Antibiotics resisted	Number of resistant strains		
		S. typhi	*S. paratyphi*	*S. arizonae*
1.	Amp. Cot.	1	0	0
2.	Amp. Cot. Chl.	1	3	1
3.	Amp. Nit. Tet.	2	1	0
4.	Amp. Chl. Tet.	1	1	1
5.	Amp. Cot. Tet.	1	1	0
6.	Amp. Chl. Tet. Str.	1	1	2
7.	Amp. Nit. Tet. Str.	3	0	0
8.	Amp. Chl. Tet. Cef.	3	0	0
9.	Amp. Chl. Tet. Cot.	1	0	3
10.	Amp. Chl. Tet. Nal.	1	0	1
11.	Amp. Chl. Cef. Nit.	5	0	0
12.	Amp. Chl. Tet. Col.	1	0	0
13.	Amp. Chl. Cot. Cef.	1	0	0
14.	Amp. Chl. Gen. Cef. Col.	4	2	2
15.	Amp. Chl. Cot. Cef. Col.	4	0	0
16.	Amp. Gen. Cot. Nit. Col.	6	1	1
17.	Amp. Str. Cot. Nit. Tet.	1	0	0
18.	Amp. Str. Chl. Nit. Cef.	2	0	1
19.	Amp. Str. Chl. Col. Tet.	1	0	1
20.	Amp. Chl. Cot. Col. Tet.	1	0	0
21.	Amp. Chl. Nit. Col. Tet.	2	0	0
22.	Amp. Chl. Nit. Cot. Tet. Str.	6	2	1
23.	Amp. Chl. Cef. Cot. Tet. Str.	1	0	0
24.	Amp. Chl. Cef. Cot. Tet. Gen.	6	0	0
25.	Amp. Cot.Cef. Nit. Tet. Gen. Col.	4	2	1
26.	Amp. Cot. Chl. Nit. Tet. Nal. Str.	1	1	0
27.	Amp. Cot. Chl. Nit. Tet. Nal. Col.	1	0	0
28.	Cef. Cot. Chl. Nit. Tet. Nal. Col. Str.	6	2	1
	Total	68	17	16

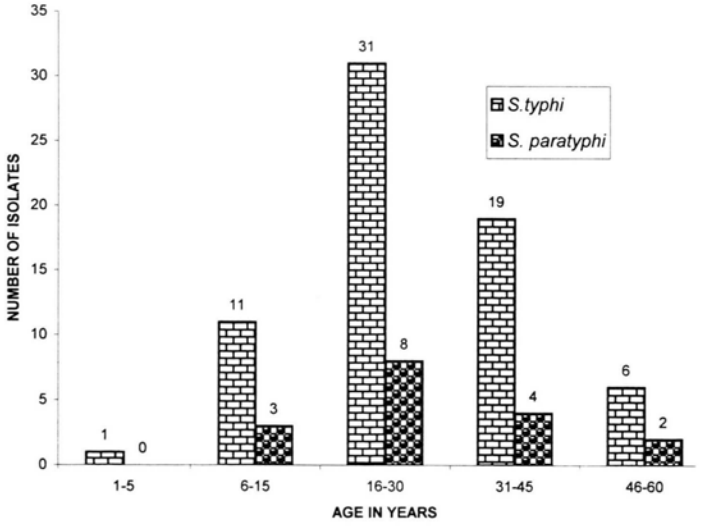

Fig. 1. Age distribution pattern of *S. typhi* and *S. paratyphi* among typhoid patients in Lagos, Nigeria.

paratyphi showed resistance to two or more of the 10 of the 12 antibiotics tested but, none developed resistance to ciprofloxacin and ofloxacin (Tables I and II).

The patients with multi-drug resistant *S. typhi* infection ranged from 1 to 60 years of age (Fig. 1) however, the sex and age distribution pattern of the resistant *S. typhi* and *S. paratyphi* is shown in Table III. Virtually all the age ranges of the patients studied yielded one or more strains of multi-drug resistant strains of the typhoid and paratyphoid bacilli.

This is the first recent report of multi-drug resistant *Salmonella typhi* from Lagos, the commercial and nerve centre of Nigeria. The increasing emergence strains of *S. typhi* to the three first-line antibiotics: chloramphenicol, ampicillin and cotrimoxazole poses a threat in developing countries since the main alternatives drugs fluoroquinolones are relatively expensive and not licensed for use in children (Adam, 1989; Arshad *et al.*, 1992). Our study showed that 10 of 12 antibiotics tested were ineffective against the *Salmonella* sp. isolated in this study (Tables I and II).

Ampicillin, chloramphenicol and cotrimoxazole were the most frequently prescribed drugs for the treatment of typhoid fever in Nigeria like other developing countries. This is because they are cheap, accessible and therefore constitute the first-line of antibiotics. Since virtually all our isolates in this study developed resistance to these three first-line antibiotics, it is therefore not improper to suggest the immediate stoppage of their prescription for the treatment of typhoid fever in Nigeria. The antibiotics found most effective among the twelve tested are ciprofloxacin and ofloxacin (the fluoroquinolones) because, none of our 101 *Salmonella* isolates developed resistance to them. Although, there have been occasional reports of *S. typhi* being resistant to ciprofloxacin and ofloxacin in some parts of the globe (Rowe *et al.*, 1992; Umasankar *et al.*, 1992), no such observation was seen in this study nor has ever been reported in Nigeria.

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Table III. Age and sex distribution of antimicrobial drugs resistant *S. typhi* and *S. paratyphi* among patients in Lagos, Nigeria.

Age in Years	PATIENTS STUDIED			DISTRIBUTION PATTERN OF ISOLATES RESISTANT TO TWO OR MORE ANTIMICROBIAL DRUGS				
	Number of Patients (%)	SEX		Total No. of Isolates	SEX		*S. typhi* (%)	*S. paratyphi* (%)
		M (%)	F (%)		M (5)	F (%)		
1–5	24 (3.8)	13 (54.2)	11 (45.8)	1 (1.2)	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)
6–15	138 (21.7)	65 (47.1)	73 (52.9)	14 (16.5)	8 (57.1)	6 (42.9)	11 (78.6)	3 (21.4)
16–30	253 (39.8)	119 (47.0)	134 (53.0)	39 (45.9)	18 (46.2)	21 (53.9)	31 (79.5)	8 (20.5)
31–45	159 (25.0)	68 (42.8)	91 (57.2)	23 (27.1)	13 (56.5)	10 (43.5)	19 (82.6)	4 (17.4)
46–60	61 (9.6)	33 (54.1)	28 (45.9)	8 (9.4)	5 (62.5)	3 (37.5)	6 (75.0)	2 (25.0)
Total	635 (100.0)	298 (46.9)	337 (53.1)	85 (100.0)	45 (52.9)	40 (47.1)	68 (80.0)	17 (20.0)

M = Male, F = Female

Number in Parenthesis represent percentages

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