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Prevalence and Susceptibility of *Salmonella typhi* and *Salmonella paratyphi* in Zaria, Nigeria

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Abstract

Purpose: To investigate the prevalence and susceptibility pattern of *S. typhi* and *S. paratyphi* isolates in Zaria.

Methods: Blood samples collected from presumptive typhoid fever patients from Ahmadu Bello University (ABU), Federal College of Education (FCE) and presumptive typhoid fever patients that attended two private clinics (Salama Clinics and Savanna Polyclinics) in Zaria were cultured for *Salmonella species* and identified by standard procedures. The susceptibility of the isolated organisms to ten antibiotics and the minimum inhibitory concentrations were determined using the methods of the National Committee for Clinical Laboratory Standards.

Results: *Salmonella species* infection rate at FCE, ABU, Salama Clinics and Savanna Polyclinic were 9.3%, 16.5%, 12.4%, and 17.2%, respectively. 102 *Salmonella species* (71 *S. typhi*, 15 *S. paratyphi* A and 16 *S. paratyphi* B) were isolated. Unlike ofloxacin and nalidixic acid, there was high resistance (60-100%) of the organisms to ampicillin, amoxicillin, Augumentin, chloramphenicol, co-trimoxazole, gentamicin, nitrofurantoin, and tetracycline. The order of susceptibility was ofloxacin > nalidixic acid > nitrofurantoin > co-trimoxazole > chloramphenicol > gentamicin > tetracycline > amoxicillin > ampicillin > Augumentin. Resistance profile of *Salmonella species* isolated from patients at ABU and FCE were significantly higher than those of the private clinics.

Conclusion: Many antibiotics previously used to treat typhoid fever are no longer effective. Laboratory tests should therefore be a major consideration in the choice of antibiotics for treatment of typhoid fever.

Keywords: Prevalence, *Salmonella species*, Antibiotics susceptibility, Resistance.

Gbonjubola O Adeshina*

Nonyelum O Osuagwu

Claire-Lorentz E Okeke

Joseph O Ehinmidu

Rebecca O Bolaji

Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria

**For correspondence*

Tel: +234-803-788-0000

Email: dotunkele@yahoo.com

Introduction

Typhoid fever or enteric fever is a systemic infection characterized by persistent high fever with low pulse rate, severe headache, toxemia, enlargement of the spleen, nausea, mental confusion, intestinal haemorrhage and perforation [1]. *Salmonella enterica* serovar *typhi*, which is the causative agent, remains an important public health problem and result to about 600,000 deaths annually worldwide [2]. A mild form the disease, paratyphoid fever, is caused by serovars paratyphi A, B and C of *Salmonella enterica* subspecies *enterica*. The bacteria is generally carried in the blood stream, intestinal tract and faecal matter of a human host and therefore, highly contagious. It can be acquired by ingestion of food and water contaminated by faeces of infected human or person to person contact. Developing countries with low level of public hygiene are frequently reported with endemic typhoid infection [1].

Salmonella enterica serovar *typhi* is now known to be markedly resistant to commonly prescribed antibiotics [3,4] and there has been increasing concern about the prevalence of multi-drug resistant *Salmonella typhi* and *Salmonella paratyphi* strains in developing countries [2,5]. There have been several reports of multi-drug resistant *Salmonella typhi* with plasmid-mediated resistance to conventional antibiotics such as chloramphenicol, co-trimoxazole and ampicillin in different parts of the world [5, 6]. Multi-drug resistant *Salmonella typhi* to antibiotic such as chloramphenicol, amoxicillin, co-trimoxazole, and fluoroquinolone have emerged as new challenges to the treatment of typhoid fever [6,7]. In Zaria in the northern part of Nigeria, anecdotal evidence indicated the need to investigate the susceptibility part of available antibiotics as the area continued to experience antibiotic failure in the treatment of typhoid fever. Hence, there is a need for the study of prevalence and susceptibility of *Salmonella species* clinical isolates to commonly prescribed antibiotics in an attempt to

understand antibiotics susceptibility profile of *Salmonella species* isolates in Zaria.

Experimental

Bacteriology

A total of 713 blood samples were collected from 713 presumptively diagnosed typhoid patients in student clinics at Ahmadu Bello University (ABU) Clinic, Federal College of Education (FCE) Clinic and two private clinics (Salama Clinics and Savanna Polyclinics) in Zaria, Nigeria within the period of December 2007 to June 2008. *Salmonella* species were isolated from the blood samples and characterized to species level using standard procedures [8].

Antimicrobial sensitivity testing

The susceptibility pattern of the isolates to selected commonly prescribed antibiotics: ampicillin (20 µg), amoxicillin (25 µg), Augmentin (30 µg), chloramphenicol (30 µg), co-trimoxazole (25 µg), gentamicin (10 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), ofloxacin (30 µg), and tetracycline (30 µg) was determined using Kirby Bauer disc diffusion method [9]. Overnight cultures of the isolated *Salmonella species* in nutrient broth were standardized to 1.0×10^6 cells/ml and flooded over the prepared Mueller-Hinton agar plates. Excesses were drained off and allowed to dry in a warm incubator for about 15–20 min. Antibiotics impregnated discs were placed on the dried inoculated agar plates aseptically. The plates were left at room temperature for 25 min to allow for the diffusion of the antibiotics into the agar medium and then incubated at 37 °C for 24 hr. Interpretation of the strains as sensitive or resistance were based on zones of inhibition according to current NCCLS standards in accordance with WHO requirements [10,11].

Results

Out of the total number of the patients (713) studied, 322 (45.2%) were female and 391

(54.8%) were male. Hundred and two (102) *Salmonella* sp. isolates were obtained from the blood samples culture. The distribution of these isolates is provided in Table 1. Majority of the culture-positive blood samples from suspected typhoid patients in this study were obtained in the early raining season. *Salmonella* species isolated from blood sample of male typhoid patients (57) was higher than isolates from blood samples of female typhoid patients (45).

Table 1: Bacterial isolates from blood samples of typhoid suspected patients in Zaria

Health facility	# of samples	# <i>Salmonella</i> isolates recovered			%
		Female	Male	Total	
ABU	304	24	26	50	16.5
FCE	162	8	7	15	9.3
Salama clinic	129	8	8	16	12.4
Savanna clinic	117	5	16	21	17.9
<i>Total</i>	<i>713</i>	<i>45</i>	<i>57</i>	<i>102</i>	<i>14.3</i>

ABU, Ahmadu Bello University Sick Bay, FCE, Federal College of Education Clinic

Table 2 shows the distribution of *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B. The analysis of the data showed that the predominant *Salmonella* species isolates in the clinics was *Salmonella typhi* accounting for 69% of the total isolates, followed by *Salmonella paratyphi* A (15%), and *Salmonella paratyphi* B (16%). Moreover, the blood samples of typhoid presumptive diagnosed students showed higher percentage of *Salmonella paratyphi* (24%) compare to that from the private clinics (14-15%).

Multi-drug resistance of *Salmonella* species isolates to some of the antibiotics including ampicillin and chloramphenicol was observed. Isolates from female presumptively diagnosed typhoid patients showed more susceptibility to ofloxacin (93.4%) than isolates from male typhoid patients (80.7%).

Table 2: Distribution pattern of *Salmonella* species isolates in the blood samples

Hospitals	# of isolates	<i>S. typhi</i> (%)	<i>S. paratyphi</i> A (%)	<i>S. paratyphi</i> B (%)
ABU	50	29 (58)	9 (18)	12 (24)
FCE	15	10 (67)	3 (20)	2 (13)
Salama clinic	16	15 (94)	0	1 (6)
Savanna clinic	21	17 (81)	3 (14)	1 (5)
<i>Total</i>	<i>102</i>	<i>71 (69)</i>	<i>15 (15)</i>	<i>16 (16)</i>

ABU, Ahmadu Bello University Sick Bay, FCE, Federal College of Education Clinic

Using zones of inhibition as yardstick for test antibiotics effectiveness, the order of effectiveness of test antibiotics was found to be ofloxacin (70.2–77.2%) > chloramphenicol (44.4–54.4%) > gentamicin (37.8–38.6%) > amoxicillin (11.1–12.3%). It was also observed that *Salmonella* species isolates showed high resistance to those antibiotics that are not frequently prescribed for typhoid treatment, such as Augmentin (91%), nitrofurantoin (57%), gentamicin (67%) and tetracycline (81%). Generally, the isolates displayed high resistance to beta-lactam antibiotics (Table 3).

Discussion

This study has reported the high level of antibiotics resistance to commonly used antibiotics for the treatment of typhoid fever in Zaria in northern Nigeria. That the majority of the culture-positive blood samples from suspected typhoid patients in this study were obtained in the early raining season has been reported by other workers [13,14]. Considering that typhoid fever is endemic and the causative agent, *Salmonella typhi*, is chiefly spread by faecal-oral route following acute infection, it is likely that the contamination of food materials or drinking water as a result of wash off from the faecal contaminated environment during early rain-

Table 3: Antibiotics resistance patterns of isolated *Salmonella* species against test antibiotics at 37 °C for 24 hr

Antibiotics	Resistance pattern (%)		
	<i>S. typhi</i> (n=71)	<i>S. paratyphi</i> A (n=15)	<i>S. paratyphi</i> B (n=16)
Amoxicillin (25µg)	63 (88.7)	12 (80.0)	16 (100)
Ampicillin (20µg)	64 (90.1)	13 (86.7)	16 (100)
Augumentin (30µg)	62 (87.3)	15 (100)	16 (100)
Chloramphenicol (30µg)	43 (60.6)	5 (33.3)	12 (75)
Cotrimoxazole (25µg)	40 (56.3)	6 (40)	10 (62.5)
Gentamicin (10µg)	42 (59.2)	12 (80)	10 (62.5)
Nalidixic acid (30µg)	29 (40.9)	7 (46.7)	8 (50)
Nitrofurantoin (300µg)	39 (54.9)	7 (46.7)	12 (75)
Ofloxacin (30µg)	7 (9.9)	5 (33.3)	5 (31.3)
Tetracycline (30µg)	56 (78.9)	13 (86.7)	14 (87.5)

ing season must have been one of the sources of typhoid fever in the studied areas. These factors could actually increase the risk of person-to-person spread especially in the school hostels. The large number of samples from ABU as compared to other areas during the sampling can be attributed to population density of the area and probably due to free medical consultation and diagnosis. However, the level of *Salmonella* species isolation from the sample was very low. Although *Salmonella* species from blood samples increases with the duration of fever from first to twenty-first day of infection [15], the indiscriminate use of antibiotics by suspected typhoid patients may probably contribute to the low level of isolation of *Salmonella* species among students [16]. The high occurrence of *Salmonella typhi* isolation in this study has earlier been reported to be due to ignorance, low level of personal, food, and water hygiene [17].

It was observed that there were no differences in percentage resistance of *Salmonella* species isolates from male patients when compared with *Salmonella* species isolates from female patients. A similar observation was reported in a study in Ile-Ife, Nigeria [18]. However, there was high susceptibility of *Salmonella* species isolates from female patients to ofloxacin than *Salmonella* species isolates from male patients.

This study reports an increase in resistance profile of *Salmonella* species isolates to commonly prescribed and non-typhoidal antibiotics. There are indications that *Salmonella* species has rapidly gained resistance to first-line antibiotics such as amoxicillin, chloramphenicol, co-trimoxazole and fluoro-quinolones [7,19,20]. The decrease in the susceptibility of the isolates to amoxicillin, ampicillin, chloramphenicol and co-trimoxazole has been reported previously [21-23]. The higher level of resistance of antibiotics to isolates from the clinics mainly attended by students as compared to the private clinics may be accounted for by higher level of self-medication by the students. Beta-lactam antibiotics showed higher resistance than other antibiotics most probably because of its frequent prescription in the area for various conditions [21]. Nalidixic acid resistance, which predicts reduced susceptibility to fluoroquinolones treatment, was observed in this study. The significant high level of resistance to ofloxacin ($p < 0.05$) by *Salmonella paratyphi* and *Salmonella typhi* isolated in this investigation may likely be due to the fact that antibiotic resistant can be transferred in-vivo from specie to specie.

Conclusion

Resistance of many antibiotics previously used to treat typhoid fever have been

reported in this study. The emergence of resistant *Salmonella* species isolates to chloramphenicol, co-trimoxazole, amoxicillin and ofloxacin calls for a policy of routine screening of antibiotics effectiveness, before prescription to reduce the spread of resistant strains.

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