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Antibiotic sensitivity profiles of salmonella organisms isolated from presumptive typhoid patients in Zaria, Northern Nigeria

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Summary
A hundred and ten Salmonella organisms (comprising 38 *Salm. typhi*, 13 *Salm. paratyphi* and 59 non-typhi Salmonella spp.) isolated from blood of patients diagnosed of typhoid fever in Zaria, Northern, Nigeria were evaluated for their *in-vitro* susceptibility to eighteen antibiotics. A high percentage of the Salmonella spp. exhibited resistance to the various test antibiotics. While ceftriaxone, ciprofloxacin and the aminoglycosides exhibited the greatest *in-vitro* activity against the isolates (82.7-96.4% of the isolates were susceptible), chloramphenicol and the aminopenicilins were considerably less effective (only 54.5% of isolates were sensitive). Multiple antibiotic resistance (MAR) indexing showed that 90.9% of the isolates had multiple drug resistance (MAR) index >0.2. Eighteen of these isolates were resistant to at least 12 of the 18 antibiotics; susceptible only to ceftriaxone, ciprofloxacin and the aminoglycosides. The percentage degree of resistance and MAR indices of the isolates to the antibiotic agents were irrespective of age and gender of the patients. The high prevalence of multiple drug resistant bacteria in this region is of epidemiological concern, as this will restrict the choices of antibiotics in the treatment of typhoid fever to a few compounds.

Keywords: *Antibiotic resistance, typhoid fever.*

Résumé
Cent dix micro-organismes de salmonella (38 *salmonella typhi*,13 *salm.paratyphi* et 59 *non-typhi* especes de salm.) étaient isolés du sang des patients diagnostiques ayant la fiévre typhoide à Zaria,une province au Nord du Nigéria et evalués par la culture de suceptibilité à 18 antibiotiques. Un grand poucentage d'espéces de salmonella demontrait la résistance aux different test d'antibiotiques. La ceftriaxone,ciprofloxacine et les aminoglycosides démontraient la plus grande activite sur ces isolats (82.7-96.4%). Le chloramphénicol et les aminopénicillines etaient non-effective. L'indexe de la multiple résistance aux antibiotiques(RMA) >0.2 était démontre sur 90% des isolats. Dix-huits des isolates étaient resistant au moins à 12 sur 18 antibiotiques sensitive seulement au ceftriaxone, cipofloxacine et aux aminoglycosides. Le degré de resistance et d'indice de multi-résistance aux agents antibiotiques étaient irrespectivement de l'age et du gendre du patient. Le taux de prevalence de la résistance multiple aux antibiotiques dans cette région est d'une concernée épidemiologique pour le choix du traitement de la fiévre typhoide.

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Introduction

Typhoid fever (an enteric fever) is a systemic infection caused by *Salm. typhi* and *Salm. paratyphi*. Though it occurs in almost all parts of the world, typhoid fever is endemic in the tropics and in particular, the third world countries. Until 1948, there was no specific drug therapy; good nursing care and patients' defensive mechanisms were relied on for recovery, hence the associated high fatality rates (30-50%) in those days [1].

Introduction of chloramphenicol in the management of typhoid in 1948 resulted in considerable reduction in fatality rates and improvement in prognosis [2]. Reports highlighting the adverse effects of chloramphenicol especially on the homeopoetic system and the emergence of high numbers of chloramphenicol resistant Salmonella isolates [3, 4, 5] led to the introduction of ampicillin/amoxycillin and co-trimoxazole in the 1970s in the therapy of typhoid fever [6]. These groups of drugs have been replaced by other drugs, due to their variability in antimicrobial activities [7] and more importantly, the emergence of resistant Salmonella organisms [5,8]. The newer Quinolones, namely norfloxacin, ciprofloxacin and ofloxacin have been introduced in the management of typhoid fever with a great degree of success [9] and are claimed to be very effective against multi-resistant Salmonella strains [10]. Ceftriaxone and cefuroxime (both cephalosporins) have also been reported to achieve very high degree of defervescence with extremely low failure rates [6].

Reports from rural areas and hospitals in eastern Nigeria showed that incidences of typhoid fever are on the increase [8]. Survey of patients' records in some health centers in Zaria, Northern Nigeria have also shown that typhoid fever is endemic in this area and epidemic outbreaks frequently occur during the early part of the rainy season [11]. This latter survey showed that reoccurrence (attributed to relapses) was very common among the populace. This study was therefore embarked upon to assess the effectiveness of the available antibiotics in the treatment of typhoid fever in Zaria with a view of re-appraising current therapy approach of typhoid fever in this part of the country.

Materials and methods

Bacteriological media

For the isolation and identification of Salmonella organisms, Bismuth sulfite, Salmonella-Shigella, and Fluid tetrathionate media (products of Oxoid Ltd, England), Deoxycholate citrate agar (E. Merck, Darmstadt, Germany), Kligler Iron agar (Becton Dickinson, Cockeysville, USA), and MacConkey agar (Biotec Laboraories, Surrey, UK) were used. Mueller Hinton agar (Oxoid Ltd, England) was used for the antibiotic sensitivity test while nutrient agar and broth media (both Oxoid products) were used for the sub-culture and maintenance of the test organisms.

Appropriate quantities of the dehydrated media were reconstituted in freshly distilled water according to the manufacturer's instructions, dispensed into respective containers and sterilized. They were kept in refrigerators until ready for use.

Isolation and identification of organisms

Blood samples were collected from patients who were presumptively diagnosed for typhoid fever. These samples were cultured into fluid tetrathionate broth. After incubation at 37°C for 24 hours, growths were subjected to microscopic examination and sub-cultured onto selective and diagnostic media such as Bismuth Sulfite, Salmonella-Shigella, MacConkey, Deoxycholate citrate and Kligler Iron agars. Biochemical tests such as citrate utilzation, catalase production, indole production, methyl red and Voges Proskauer, sugar fermentation, urease production, oxidase and hydrogen sulphite production, were carried out as recommended [12,13].

Isolates that possessed characteristic morphological and biochemical properties of Salmonella organisms [14] were further differentiated to species level (*Salm. typhi*, *Salm. paratyphi*, non-typhi Salmonella), based on specific differentiation properties given in API 20 monograph [15]. Pure cultures were maintained on nutrient agar slants for subsequent use.

Determination of in-vitro antibiotic sensitivity of isolates

Susceptibility of the Salmonella isolates to various antibiotics was determined using the agar diffusion method as described by the National Committee on Clinical Laboratory services[16]. Discs of ampicillin (10μg), azlocillin (30μg), cefaclor (30μg), ceftriaxone (30μg), chloramphenicol (30μg), ciprofloxacin (30μg), erythromycin (15μg), kanamycin (30μg) and tobramycin (10μg) (from Becton and Dickinson GmBH, Heidelberg, Germany); Augmentin^R (Smith-Kline Beecham GmBH, Munchen, Germany); doxycycline 30μg (Pfizer GmBH, Karlsruhe, Germany), gentamicin 10μg (E. Merck, Darmstadt, Germany), nitrofurantoin 100ug (Rohm Pharm GBH Weiterstadt, Germany); piperacillin 30μg (Lederle Arzneimittel, GmBH, Walfratshausen, Germany) and vancomycin 30μg (Lilly Deutschland GmBH, Giessen, Germany) were used. Pure powders of sulphamethoxazole and trimethoprim (National Institute for Pharmaceutical Research and Development, Abuja, Nigeria) and Septrin^R syrup (Wellcome Foundation SKG Ltd, Lagos, Nigeria) were also utilized in the sensitivity test.

Overnight nutrient broth cultures of the Salmonella isolates were standardized with sterile 0.9%w/v normal saline to contain approximately 10^5 cfu/ml. Using sterile cotton swabs, agar surfaces of Mueller Hinton medium in sterile petri-dishes were inoculated with the salmonella organisms. Antibiotic impregnated discs were applied on the inoculated agars with the aid of a multi-disc dispenser, left at room temperature for antibiotic pre-incubation diffusion time of one and a half hours. Thereafter, the agar plates were incubated at 37°C for 24 hours.

Agar well diffusion method was employed in case of the anti-metabolites (co-trimoxazole, sulphamethoxazole and trimethoprim). Standardized overnight cultures of the Salmonella isolates were mixed with melted Mueller Hinton agar at 45°C (to contain *ca* 10^5 cfu/ml) and poured into sterile plates to set. Using a cork-borer of 8.00mm diameter wells were bored in the agars, and filled with 0.2ml of aqueous suspensions of the antibiotics (equivalent to 25μg of co-trimoxazole, 100μg of sulphamethoxazole or 5μg of trimethoprim). After pre-incubation diffusion time of 2 hours, the plates were incubatedat 37°C for 24 hours.

For each isolate, triplicate samples were prepared per antibiotic. After incubation, diameters of zones of inhibition were measured. Interpretation of zones sizes in term of susceptible, moderately susceptible and resistant was based on the values provided by the National Committee on Clinical Laboratory Services [16] and The American Hospital Formulary Services (AHFS) Drug Information 90 [17]. The multiple antibiotic resistance (MAR) index for each Salmonella isolate was calculated using the formula recommended by Krumpermann [18]:

$$\text{MAR} = \frac{\text{No of antibiotics to which organism is resistant}}{\text{No of antibiotics to which organism is exposed}}$$

Results and discussion

A total of 110 Salmonella organisms were isolated from the 315 blood samples obtained from presumptively diagnosed typhoid patients. Of this number, 38 were confirmed as *Salm. typhi*, 13 as *Salm. paratyphi* and the remaining 59 as non-typhi Salmonella.

The interpretation of the zones sizes of inhibition produced by the test antibiotics on the *Salm. typhi* and *Salm. paratyphi* isolates as shown in Table 1 indicates that ceftriaxone, ciprofloxacin and the aminoglycosides (gentamicin, kanamycin and tobramycin) exhibited the greatest in-vitro antimicrobial activities against the typhoid causative organisms. Percentage susceptibility among the isolates ranged from 90.20% for kanamycin to 98.04% for ceftriaxone and ciprofloxacin. Chloramphenicol, a reserved

drug in the therapy of typhoid fever infection was considerably less effective, inhibiting approximately 55% of the isolates, an activity similar to those exhibited by ampicillin and amoxycillin-clavulanic acid. Co-trimoxazole and trimethoprim (both anti-metabolites) were relatively less effective, inhibiting only 47% of the isolates. A high number of the isolates (80-92%) were resistant to erythromycin and vancomycin. This antibiotic sensitivity pattern is in agreement with observations of some previous workers [19] on the sensitivity profiles of *Salm. typhi* and *Salm. paratyphi* isolated from blood and bone marrow of typhoid patients.

Table 1: Antibiotic sensitivity profiles of *Salm. typhi* and *Salm. paratyphi* organisms isolated from the patients (n = 51)

Antibiotic	No of organisms			%
	Sen-sitive	MS	Resis-tance	Resis-tance
Penicillins:				
Ampicillin (10ug)	20	7	24	47.06
Amoxy-Clav (20/10 ug)	17	3	31	60.78
Azlocillin (30ug)	16	8	27	52.94
Piperacillin (30ug)	19	11	21	41.18
Cephalosporins:				
Cefaclor (30ug)	12	6	33	64.71
Ceftriaxone (30ug)	41	9	1	1.96
Aminoglycosides:				
Gentamicin (10ug)	40	8	3	5.88
Kanamycin (30ug)	45	1	5	9.80
Tobramycin (30ug)	39	8	4	7.84
Antimetabolites:				
Co-trimoxazole (25ug)	24	0	27	52.94
Trimethoprim (5ug)	20	4	27	52.94
Sulphamethoxazole (100ug)	7	7	37	72.55
Ciprofloxacin (5ug)	48	2	1	1.96
Chloramphenicol (30ug)	16	12	23	45.10
Doxycycline (30ug)	9	10	32	62.75
Nitrofurantoin (100ug)	12	6	33	64.71
Erythromycin (15ug)	4	1	46	90.20
Vancomycin (30ug)	1	9	41	80.39

MS = moderate susceptibility

The high percentage of sensitivity of the isolates to ceftriaxone, ciprofloxacin and the aminoglycosides may not be unconnected with their low level of use. Ciprofloxacin and other new quinolones recently introduced into the Nigerian drug market, are relatively costly and hence not widely in use. Ciprofloxacin and the new quinolones have been found very useful in the treatment of multi-resistant strains of *Salm. typhi* and *Salm. paratyphi* A. For example, Mirza *et al* [10] reported in 1995 that 91% of patients with multi-resistant *Salm. typhi* in Pakistan achieved defervescence within 3 days on treatment with fluoroquinolones while the remaining 9% took 4-6 days for defervescence to occur. Ceftriaxone, a third generation cephalosporin, is one of the most expensive antibiotics in the Nigerian drug market and available only as a parenteral; factors which have restricted its use to the management of severe infections and therefore, is hardly abused. Availability of aminoglycosides only in injectable form may also be responsible for its non-abuse. These three categories of drugs were hardly prescribed by medical practitioners in the management of typhoid therapy in Zaria [11]. The observed high percentage of the *Salm. typhi* and *Salm. paratyphi* isolates resistant to chloramphenicol, amoxycillin, ampicillin and co-trimoxazole observed in this study agrees with the results of other workers in many parts of the world [3,4,5,19,20,21,22,23]. A similar study [8] carried out in the eastern part of Nigeria also showed high percentage of resistance among Salmonella strains to these antibiotics.

Table 2: Antibiotic sensitivity profiles of the non-typhi Salmonella organisms isolated from the patients (n = 59)

Antibiotic	No of Organisms			%
	Sen-sitive	MS	Resis-tance	Resis-tance
Penicillins:				
Ampicillin (10ug)	20	11	28	47.46
Amoxy-Clav (20/10 ug)	24	5	30	50.85
Azlocillin (30ug)	24	5	30	50.85
Piperacillin (30ug)	28	6	25	42.37
Cephalosporins:				
Cefaclor (30ug)	24	4	31	52.54
Ceftriaxone (30ug)	48	7	4	6.78
Aminoglycosides:				
Gentamicin (10ug)	42	10	7	11.86
Kanamycin (30ug)	41	4	14	23.73
Tobramycin (30ug)	41	13	5	8.47
Antimetabolites:				
Co-trimoxazole (25ug)	17	0	42	71.19
Trimethoprim (5ug)	14	1	44	74.58
Sulphamethoxazole (100ug)	6	7	46	77.97
Ciprofloxacin (5ug)	54	2	3	5.08
Chloramphenicol (30ug)	21	12	26	44.07
Doxycycline (30ug)	11	12	36	61.02
Nitrofurantoin (100ug)	17	3	39	66.10
Erythromycin (15ug)	5	0	54	91.53
Vancomycin (30ug)	3	1	55	93.22

MS = Moderately susceptible

This may be due to the fact that these antibiotics are the most frequently prescribed drugs in the therapy of typhoid infections in this environment and in most cases, antibi-

otic sensitivity tests were hardly performed [11]. The high resistance of isolates to drugs such as cefaclor, erythromycin and vancomycin is not unexpected as these drugs are intrinsically ineffective against most gram-negative organisms, including the causative agent of typhoid fever.

As shown in Table 2, the pattern of distribution of susceptibility/resistance of the non-typhi Salmonella closely resembles those of the typhoid bacteria presented in Table 1. Ceftriazone, ciprofloxacin and the aminoglycosides also exerted the greatest inhibitory activities against the non-typhi Salmonella isolates, though with slightly lower susceptibility figures: from 76.30% (kanamycin) to 95% (ciprofloxacin). The isolates were also comparably more resistant to the inhibitory effects of vancomycin, co-trimoxazole and trimethoprim. The percentage susceptibility values of the non-typhi Salmonella isolates to the other test antibiotics were also very similar to the figures obtained with the typhoid bacteria isolates. The isolation of non-typhi Salmonella spp. from patients presumptively diagnosed of typhoid fever is in consonance with the report of a similar study carried out in Korea [24].

Table 3: Distribution of the antibiotic resistances of the Salmonella isolates according to the age groups of the patients

Antibiotics	% Resistance		
	Adult	Children	Population Average
Amoxycillin-clavulanic acid	55.42	55.56	55.46
Chloramphenicol	43.78	46.45	45.45
Co-trimoxazle	64.71	47.62	62.73

Table 3 shows the comparative antibiotic susceptibility profiles of all the Salmonella organisms based on the ages of the patients. Responses to four antibiotics representing different classes and degree of susceptibilities as indicated in this table, showed that there are virtually no differences in the percentage resistances of the isolates that could be attributed to age differences of the patients except with co-trimoxazole in which a higher percentage of the Salmonella organisms isolated from adults were resistant to the drug.

The MAR indices depicted in Table 4 shows that high percentage of the organisms exhibited multiple antibiotic resistances (i.e. have been exposed to several antibiotics). One hundred of the 110 isolates (92% of the 51 typhoid bacteria and 90% of the non-typhi Salmonella spp.) have MAR index ≥ 0.2, i.e. resistant simultaneously to more than four antibiotics. The ten isolates with MAR indices of < 0.2 were resistant only to erythromycin, vancomycin, sulphamethoxazole and either nitrofurantoin or trimethoprim. Only one isolate, obtained from a female patient was sensitive to all the eighteen antibiotics. Eighteen of the Salmonella isolates (6 of the typhoid bacteria and 11 of the non-typhi Salmonella) were exceptionally highly multiple drug resistant, exhibiting resistances to at least twelve of the eighteen antibiotics.

Table 4: Multiple antibiotic resistance (MAR) indices of the Salmonella isolates (n=110)

MAR Index	Frequency		
	Salm. typhi & *Salm. paratyph*	non-typhi Salmonella spp	Total
0.0	1 (1.96)	0	1 (0.9)
0.1	2 (3.92)	1 (1.69)	3 (2.7)
0.2	1 (1.96)	5 (8.47)	6 (5.5)
0.3	10 (19.61)	6 (10.95)	16 (14.6)
0.4	10 (19.61)	17 (28.81)	27 (24.5)
0.5	9 (17.65)	6 (10.95)	15 (13.6)
0.6	12 (23.53)	12 (20.34)	24 (21.8)
0.7	6 (11.76)	7 (11.86)	13 (11.8)
0.8	0	5 (8.47)	5 (4.5)
Total	51	59	110

**Values in parenthesis are the percentage values*

Table 5: Distribution of the Salmonella Isolates with Low and high MAR indices according to age and gender

Age/Gender	No. of Isolates	Frequency at MAR Index of	
		≤0.2 (low)	≥0.7 (high)
Age:			
Adult	87	8 (9.20%)	12 (13.75%)
Children	23	2 (8.70%)	6 (26.10%)
Gender:			
Male	50	4 (8.00%)	8 (16.00%)
Female	60	6 (10.00%)	10 (16.67%)

**Values in parenthesis are the percentage figures based on the total number of isolates in each category (as shown in column 2)*

Analysis of the MAR index distribution according to age and gender showed that there might not be any relationship between gender and level of multiple drug resistance (Table 5). For example, percentage of isolates with MAR < 0.2 is 8% for males compared with 10% for females while at high MAR indices (MAR> 0.7), the percentages are 16% for male and 16.7% for female patients. When the MAR indices were compared on the basis of age, a relatively higher percentage of Salmonella isolates obtained from young patents (< 15 years), were highly

multiple drug resistant. Approximately 26% of isolates obtained from young patients (6/23) had MAR index >0.7 compared with 13.75% of isolates from adult patients (12/87). At low MAR index values (<0.2), the percentages were however similar (8.7% Vs 9.2%). Usually MAR index greater than 0.2 is an indication that the strain originates in an environment where several antibiotics are in use [19]. The isolation of typhoid bacteria and non-typhoid Salmonella sp. with multiple antibiotic resistances in this work is not new. As far back as 1964, the emergence of Salmonella organisms resistant simultaneously to chloramphenicol, tetracycline and ampicillin was reported [25]. *Salm. typhi* simultaneously resistant to chloramphenicol and co-trimoxazole was also reported in 1981 [26] while a study carried out in 1986 [27] found that 29.9% of isolates of *Salm. typhi* were resistant simultaneously to chloramphenicol, ampicillin, sulphonamides and trimethoprim.

The high multiple antibiotic resistance indices of the Salmonella organisms as revealed in this study may be due to prevailing poverty, drug misuse and abuse. In a study on the carriage of resistant strains of *E. coli* among healthy children, it was discovered that children in third world countries are greater carriers of multi-resistant organisms [28]. The prevalence of MAR bacteria isolates in the third world countries was attributed to the low level of personal and social hygiene and greater exposure to enteric flora infections. This high MAR indices may explain the frequent failure rate in the management of typhoid fever in Zaria. In this part of the country and indeed, all over Nigeria, there is virtually no enforcement of regulations governing drug distribution and use. Antibiotics are readily available from several illegal outlets, namely patent medicine sores, street drug vendors and open markets. A large proportion of the populace hardly patronizes qualified medical practitioners because of unaffordable medical bills. They therefore resort to self-medication, patronize medical and paramedical quacks with its attendant risks and implications. These practices are more likely to account for the prevalence of multiple antibiotic resistances among the isolates in this environment than the use of antibiotics in animal feeds and husbandry as postulated by some workers [29,30]. The high prevalence of MAR bacteria in this region is of epidemiological concern, as this will restrict the choices of antibiotics in the treatment of typhoid fever to a few compounds. It also calls for caution in the indiscriminate use of antibiotics, and re-appraisal in the current approach and management of typhoid fever in this part of the country.

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