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### THE AETIOLOGY OF ENTERIC FEVER IN ABUJA, NORTH CENTRAL NIGERIA

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#### ABSTRACT

Enteric fever is caused by *Salmonella enterica serotype typhi*, *Salmonella paratyphi* A, B, C, and *Salmonella typhimurium* respectively. Of the 2818 blood cultures reviewed, only 90 (3.2%) had positive cultures for *Salmonella* species while the 10,007 faecal samples cultured, 159 (1.6%) were positive for *Salmonella* species. Identification of isolates was by usual bacteriological techniques including biochemical and serological methods. Percentage occurrence of *Salmonella* species in blood and faecal samples show *Salmonella enterica serotype typhi* (75.6% and 59.8%), *Salmonella paratyphi* A (4.4% and 9.4%), *Salmonella paratyphi* B (17.8% and 19.5%), *Salmonella paratyphi* C (2.2% and 6.3%) and *Salmonella typhimurium* (0.0% and 5.0%). The susceptibility pattern of all the isolates to the eleven drugs used as listed on table iii is highly revealing. For epidemiological status and proper management of patients, it is necessary that appropriate specimens (blood, bone marrow and faecal cultures) are examined and identification of isolates carried out as well as proper sensitivity testing performed prior to treatment for enteric fever.

Keywords: Enteric fever, Blood, Faecal cultures, *Salmonella* species, Percentage occurrence.

#### INTRODUCTION

Enteric fever is caused by *Salmonella enterica serotype typhi*, *Salmonella paratyphi* A, B, C, and *Salmonella typhimurium* respectively. Typhoid fever (infection) is an important global health problem with an estimated 16 million cases and 216,000 - 600,000 or more deaths each year, placing typhoid fever in the range of several priority infectious diseases, including *Human Papilloma Virus* (HPV), rotavirus and *Haemophilus influenza* Type b (Hib). Very high rates of typhoid incidence were found in several DOMI (Diseases of the Most Impoverished) sites, providing further evidence that typhoid continues to be a serious problem (1). It is endemic worldwide but most cases occur in areas of Africa, Asia, and Latin America where sanitation is poor. Although no longer prevalent in the developed world, the aetiological agents continue to cause enteric fever in many parts of the developing world, especially in Asia and northern regions of Africa (2). In 2004, these agents were still estimated to cause approximately 22 million cases of disease and 200,000 deaths each year, primarily in regions where sanitation is poor and clean water is inaccessible (3).

*Salmonella enterica serotype typhi* is the aetiological agent of typhoid fever, a multisystem disease with protean manifestations and initial lesions in the bowel. *Salmonella paratyphi* A & B cause paratyphoid fever.

Typhoid fever still remains a major public health problem in developing countries even in the twenty first century (4, 5). In Nigeria, as in other developing countries of the world, studies have estimated over 33 million cases and 500,000 deaths due to typhoid fever per year (6). Several factors have been attributed to the failure of public health measures to tame the tide of the continuing rise in the incidence, prevalence, morbidity, and mortality of typhoid fever.

It is customary in our society that any feverish condition is first treated for malaria. If this fails, then treatment for typhoid automatically follows and if the patient at this stage fails to respond, it is only then that laboratory investigations are remembered (7). Salmonellosis is responsible for a variety of clinical syndromes including gastroenteritis, enteric (typhoid) fever and extraintestinal manifestations.

Typhoid fever remains one of the most prevalent acute infectious diseases in the developing world including Nigeria. It continues to exist as an endemic disease due to poor (improper) sanitation and low socio-economic status of the people (7).

The clinical diagnosis of typhoid fever is considered to be unreliable (8). A definite diagnosis is obtained when the aetiological agent, *Salmonella enterica serotype typhi* is isolated from faeces, blood, or bone marrow (9).

The aim of this work is to re-emphasize the need to properly identify aetiological agents for enteric fever in our region prior to treatment of same.

## MATERIALS AND METHODS

2818 blood cultures and 10007 faecal samples at the Medical Microbiology Laboratory of National Hospital, Abuja was studied.

## Blood Culture

The Oxoid Signal Blood Culture System (produced by Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, England) was used to culture samples of

blood collected from patients where the condition of bacteraemia is suspected.



FIGURE I: PICTURE OF OXOID SIGNAL BLOOD CULTURE SYSTEM

### Collection and Processing of Blood Samples (Blood Culture)

10 ml of blood collected from patient is inoculated into Oxoid Signal Blood Culture bottle. This is a semi-automated system that recognizes bacterial growth in the blood culture by gas production. The inoculated bottle is placed (incubated) at 36°C (+/-) 1°C for 1 hour before inserting the signal device. It is continuously shaken for 24 hours. Incubate at 36°C (+/-) 1°C for at least 7 days (according to manufacturer's instruction).

A positive Oxoid blood culture bottle is indicated by upward movement of fluid into the signal device while a negative Oxoid blood culture bottle is indicated by absence of fluid in the signal device.

All positive bottles are sub-cultured onto Chocolate agar, 3 Blood agar, and MacConkey agar plates and incubated for 24 hours at 36°C (+/-) 1°C. When applicable, it is re-incubated for a further 18 - 24 hours. The second Blood agar plate is incubated at 10% CO<sub>2</sub> enriched environment while the third Blood agar plate is incubated anaerobically (AnO<sub>2</sub>) for 48 hours. In 2000,

it was stated that in typhoid, *Salmonella enterica serotype typhi* can be detected in the blood of 75 - 90% of patients during the first 10 days of infection and in about 30% of patients during the third week (10).

Bacterial isolates were identified by Gram stain, biochemical reactions (KIA, urease test, citrate utilization test), motility test (11). Implicated bacterial isolates by way of the above identification methods were confirmed by sero-typing using *Salmonella* polyvalent O and H antisera and monovalent A, B, C, and D sera. The Vi sera is also available for typing (virulence factor).

Antibiotic susceptibility (sensitivity) test using the disc diffusion technique is then carried out with appropriate drugs on confirmed *Salmonella* isolates.

### Collection and Processing of Faecal Samples

3 - 10 grams of faeces collected over several days (usually 3 days) are preferred (12). However, we made use of only one sample. Faecal samples were cultured

on Salmonella/Shigella Agar (SSA) or Deoxycholate Citrate Agar (DCA) and Selenite Fluid (SF) and incubated at 37°C for 18 - 24 hours. The Selenite fluid preparation is sub-cultured on SSA or DCA and further incubated at 37°C for 18 - 24 hours.

*Salmonella* and *Shigella* species are non lactose fermenters. All non lactose fermenting colonies (NLFs) isolated are subjected to identification as stated above

under the blood culture methodology. Identified and confirmed *Salmonella* isolates are then subjected to antibiotic susceptibility test using the disc diffusion technique with appropriate antibiotics discs.

**RESULTS**

A total of 2818 blood cultures were examined, but only 90 (3.2%) had positive cultures for *Salmonella* species. The species identified is as stated below;

TABLE I: OCCURRENCE OF SALMONELLAE SPECIES FROM BLOOD:

<u>Species</u>	<u>Number of Isolates</u>	<u>% Occurrence</u>
<i>Salmonella enterica serotype typhi</i>	68	75.6%
<i>Salmonella paratyphi A</i>	4	4.4%
<i>Salmonella paratyphi B</i>	16	17.8%
<i>Salmonella paratyphi C</i>	2	2.2%
<b>Total</b>	<b>90</b>	<b>100.0%</b>

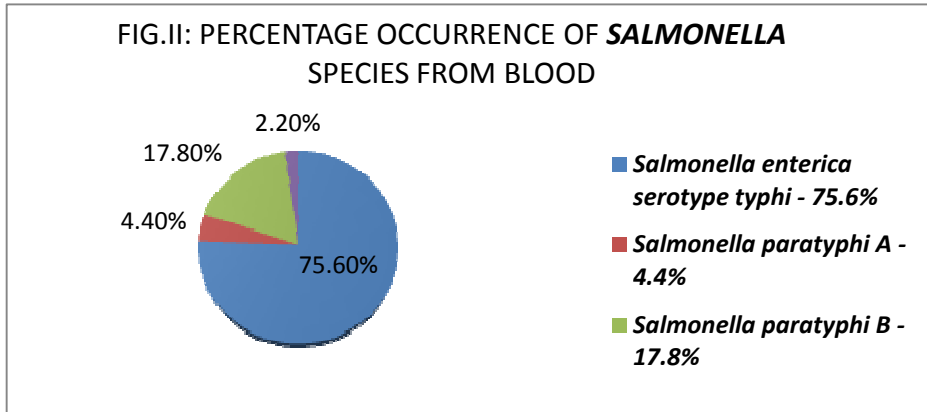


TABLE II: OCCURRENCE OF SALMONELLAE SPECIES FROM FAECES

<u>Species</u>	<u>Number of Isolates</u>	<u>% Occurrence</u>
<i>Salmonella enteric serotype typhi</i>	95	59.8%
<i>Salmonella paratyphi A</i>	15	9.4%
<i>Salmonella paratyphi B</i>	31	19.5%
<i>Salmonella paratyphi C</i>	10	6.3%
<i>Salmonella typhimurium</i>	8	5.0%
<b>Total</b>	<b>159</b>	<b>100.0%</b>

## Faecal Cultures Result

A total of 10,007 faecal samples were cultured, but only 159 (1.6%) had positive cultures for *Salmonella* species.

The species identified is as stated below;

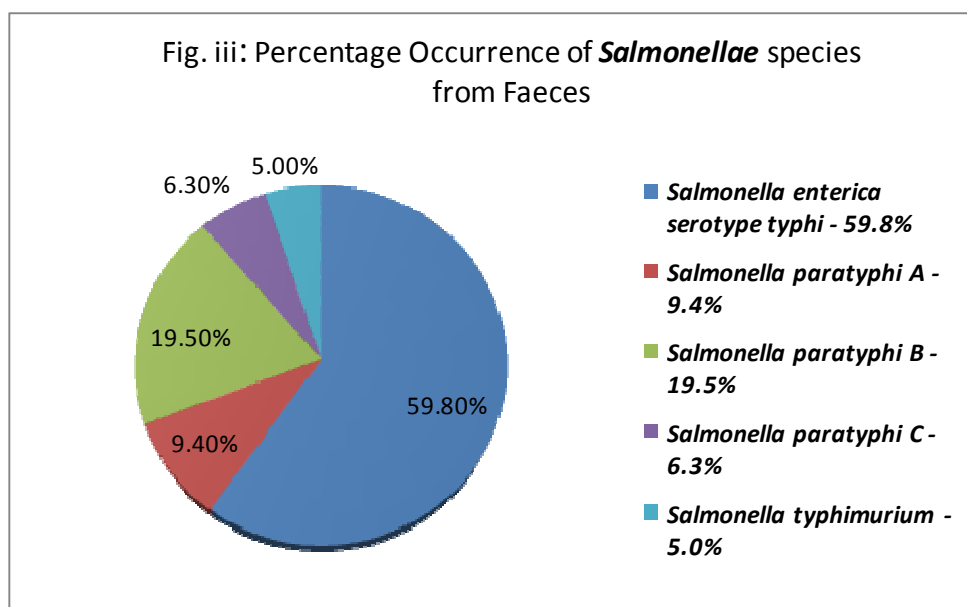


TABLE III: SUSCEPTIBILITY PATTERN OF SALMONELLAE SPECIES TO ALL DRUGS

Drugs	<i>S. typhi</i>			<i>S. para A</i>			<i>S. para B</i>			<i>S. para C</i>			<i>S. typhimurium</i>		
	NT	NS	%S	NT	NS	%S	NT	NS	%S	NT	NS	%S	NT	NS	%S
Amoxicillin	63	34	54%	9	3	33.3%	20	11	55%	6	2	33.3%	1	1	100%
Ampicillin	30	9	30%	5	2	40%	11	4	36.4%	3	1	33.3%	2	1	50%
Augmentin	129	101	78.3%	10	6	60%	35	18	51.4%	8	6	75%	7	5	71.4%
Cefotaxime	36	34	94.4%	7	7	100%	23	22	95.7%	3	3	100%	4	4	100%
Ceftazidime	50	49	98%	8	8	100%	29	28	96.6%	7	7	100%	8	8	100%
Ceftriaxone	63	60	95.2%	12	11	91.7%	30	30	100%	8	8	100%	3	3	100%
Chloramphenicol	135	81	60%	11	6	54.5%	38	28	73.7%	8	7	87.5%	1	1	100%
Ciprofloxacin	49	48	98%	7	7	100%	20	20	100%	5	5	100%	2	2	100%
Cotrimoxazole	53	36	68%	9	4	44.4%	21	12	57.1%	7	4	57.1%	6	4	66.7%
Gentamicin	63	53	84.1%	7	5	71.4%	32	24	75%	7	4	57.1%	3	3	100%
Tetracycline	51	29	56.9%	10	4	40%	22	10	45.5%	8	5	62.5%	2	2	100%

Note: *S. typhi* = *Salmonella enterica serotype typhi*;

*S. para B* = *Salmonella paratyphi B*

*S. para A* = *Salmonella paratyphi A*;

*S. para C* = *Salmonella paratyphi C*

*S. typhimurium* = *Salmonella typhimurium*

NT = Number tested against drug; NS = Number susceptible; % S = Percentage Susceptibility

## DISCUSSION

The diagnosis and treatment of enteric fever based on signs and symptoms with a “positive” Widal test will continue to be a subject of controversy (13). In 1986, Edelman & Levine (8) stated that the clinical diagnosis of typhoid fever is considered to be unreliable. Given the diagnostic inadequacies of the Widal test, the use of blood and faecal culture is recommended when suspecting enteric fever (14). Moreover, modern medicine relies on “evidence based medical practice” which is embedded in the actual isolation, identification, and susceptibility patterns of the aetiological agents from the microbiology laboratory.

In 1984, Hoffman, *et al.*, (9) stated categorically that a definitive diagnosis of typhoid fever is obtained when the aetiological agent *Salmonella enterica serotype typhi* is isolated from faeces, blood, or bone marrow.

Of the 2,818 blood cultures in this study, only 90 (3.2%) had positive cultures for *Salmonella* species. Of the 90 isolates, 68 (75.6%) were *Salmonella enterica serotype typhi*, *Salmonella paratyphi* A, 4 (4.4%), *Salmonella paratyphi* B, 16 (17.8%) and *Salmonella paratyphi* C, 2 (2.2%).

Of the 10,007 faecal samples cultured in this study, only 159 (1.6%) had positive cultures for *Salmonella* species. Of the 159 isolates, 95 (59.8%) were *Salmonella enterica serotype typhi*, *Salmonella paratyphi* A, 15 (9.4%), *Salmonella paratyphi* B, 31 (19.5%), *Salmonella paratyphi* C, 10 (6.3%), and *Salmonella typhimurium*, 8 (5.0%).

In this study, the isolation rate of *Salmonella enterica serotype typhi* was higher from blood cultures than from faecal cultures (75.6% from blood and 59.8% from faeces). The reverse was however the case with the other species. Of the *Salmonella paratyphi* A isolates, blood cultures had 4.4% as against the 9.4% from faecal

cultures. Blood cultures had 17.8% of *Salmonella paratyphi* B isolation rate as against the 19.5% from faecal cultures. 2.2% of *Salmonella paratyphi* C were recovered from blood cultures as against the 6.3% from faecal cultures. We have no explanations why the above trend was encountered. However, the isolation of *Salmonella typhimurium* from faecal samples only was not surprising. In 2007, it was reported that blood cultures are usually negative for *Salmonella typhimurium*, but faecal cultures remain positive for several weeks after clinical recovery (15).

## Conclusion

The laboratory diagnosis or confirmation of enteric fever entails the isolation of the causative (aetiological) agent and the determination of its sensitivity or resistance patterns to commonly used antimicrobial drugs for proper patient management.

The indiscriminate pretreatment with antibiotics before seeking medical attention in our hospitals and subsequent actual laboratory diagnosis continues to reduce the recovery rate of *Salmonellae* from our patient population.

Most times, the clinician may not wait to carry out laboratory test before commencement of treatment. This should be seen and viewed as contrary to modern medical practice which is “evidence based”. Moreover, this impatient attitude by some medical practitioners among other negative effects will certainly increase the rate of bacterial resistance to commonly used antibiotics.

In conclusion therefore, treatment for enteric fever should henceforth be through antibiotics (drugs) dispensed at the Pharmacy based on the Clinician’s prescription which rely on proper laboratory diagnostic result clearly stating the aetiology and susceptible drugs

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