EPIDEMIOLOGY OF TYPHOID FEVER IN ZARIA

Thesis Submitted to the Postgraduate School in Partial fulfilment of the Requirements for the Award of the Degree of Master of Science

BY

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JUNE, 1997

DECLARATION

This is to certify that the work presented in this thesis was carried out by me under the supervision of Dr. J.B. Ameh and Dr. J.K.P. Kwaga in the Laboratory of the Department of Microbiology, Ahmadu Bello University.

No part of this work has been presented elsewhere for a degree or diploma. The work of other investigators is acknowledged and referred to in this presentation.

Ahmadu Bello University, Zaria
June, 1997

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CERTIFICATION

The thesis entitled "Epidemiology of Typhoid Fever in Zaria" by Eucharia, E. Nmema meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

Dedicated to my father, Innocent John Nmema and my mother Rosanna Nmema.

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ABSTRACT

A total of 500 patients attending various hospitals in Zaria were examined for S. typhi and its '0' and 'H' agglutinins over a period of six months from May to October 1996. They consisted of 249 males and 251 females whose ages ranged between 1 and 65 years. Clinical samples collected from the patients consisted of 500 blood, 382 stool and 118 urine specimens. Widal agglutination tests were carried out with the blood samples, while stool and urine samples were cultured for isolation of S. typhi.

Eighty-two (16.4%) blood samples gave positive Widal agglutination reactions with titres of sl60 against the S. typhi 'O' antigen and 136 (27.2%), against the 'H' antigen.

Salmonella typhi and S. paratyhi were isolated from thirty-six (7.2%) of the stool and urine samples. Twenty-three (4.60%) of the isolates were identified as S. typhi. while thirteen (2.60%) were identified as S. paratyphi.

During the six months study period, the highest prevalence of typhoid fever occurred in August (12.7%), followed by May (11.1%).

In the age groups, the highest isolation frequency was in the 11-20 years age group (15.0%), followed by the 21-30 years age group (11.8%) , while the male to female ratio among those infected was 1.4:1.0.

Among the various occupations, the traders and farmers had the highest prevalence of typhoid fever with 9.67% and

9.43% respectively.

The cases of typhoid and paratyphoid fevers who had access to pipeborne water were thirteen (5.0%) and those that obtained their drinking water from wells were twenty-three (10.5%) . Fourteen (4.6%) of the cases used water closet toilets while twenty-two (11.1%) used pit latrines.

Of the 500 patients sampled, 280 had started self-medication with various antimalarial drugs and thirty-three had started on a course of chloramphenicol or Amoxicillin before attending hospital.

Only two of the 500 patients had been previously vaccinated against typhoid fever.

All the twenty-three S. typhi isolates were sensitive to five of the antibiotics namely, Chloramphenicol, Gentamicin, Ceftazidime, Ampicillin-Sulbactam and Co-trimoxazole.

The minimum inhibitory concentration of Chloramphenicol, Ampicillin-Sulbactam and Co-trimoxazole for the S. typhi. isolates ranged between 3.9u/ml to 15.6u/ml.

TABLE OF CONTENTS

Content	Page
Title page	i
Declaration	ii
Certification	iii
Dedication	iv
Acknowledgement	v
Abstract	vii
Table of Contents	ix
List of Tables	xii
List of figures	xiii
List of Appendices	xiv
Chapter One: Introduction.....	1
Chapter Two: Review of Literature	
2.1 Historical Review	5
2.2.1 The genus Salmonella	6
2.2.2 Cultural Characteristics	7
2.2.3 Biochemical Properties	8
2.2.4 Classification	13
2.2.5 Antigens of Salmonella	17
2.2.5.1 The Kauffmann - White Scheme	19
2.2.5.2 Antigenic Variation	21
2.2.5.3 Serological typing	22
2.3 Epidemiology	22
2.3.1 Distribution	23
2.3.2 Salmonella typhi and the Environment	25

2.3.3 Sources of Infection during Outbreaks of typhoid fever 29

2.3.4 Typing methods in Epidemiological Analysis of S. typhi infection 30

2.3.5 Susceptibilities of Salmonella to Physical and Chemical Agents 31

2.3.6.1 Incidence of Typhoid Fever 32

2.3.6.2 Seasonal Variation 33

2.3.6.3 Age - Sex Incidence 33

2.3.6.4 Socio-economic factors 34

2.4.1 Pathogenesis 35

2.4.2 Clinical presentation 38

2.4.3 Complications 39

2.4.4 Carrier Rate 42

2.5 Diagnosis 42

2.6 Therapy of Typhoid Fever 47

2.7 Antibiotic Sensitivity 49

2.8 Control of Typhoid Fever 51

Chapter Three: MATERIALS AND METHODS

3.1 Hospital-Based sampling and Screening..... 55

3.1.1 Questionnaires 55

3.1.2 Widal Agglutination Tests 55

3.2 Bacteriological Cultural for the Isolation of S. typhi 57

3.2.1 Selective Enrichment 57

3.2.2. Selective and Differential Plating 58

3.3 Biochemical Characterization 58

3.4 Serological Tests 59

3.5 Biochemical Differentiation of Salmonella Serotypes 59

3.6 Antimicrobial Susceptibility Testing 59

3.7 Determination of Minimum Inhibitory Concentration (MIC) 61

Chapter Four: RESULTS

4.1 Screening/Sampling of Patients 63

4.1.1 Widal agglutination tests 63

4.1.2 Biochemical Characterization of Isolates 63

4.2 Results of Serological typing 64

4.3 Biochemical Differentiation of Salmonella Serotypes 64

4.4 Specific Rates 65

4.4.1 Monthly Frequency 65

4.4.2 Age and Sex - Specific Rates 65

4.4.3 Socio-economic Status 66

4.5 Disease experience 67

4.6 Susceptibility Pattern of S. typhi Isolates 67

Chapter Five: Discussion 87

References 96

Appendices 108

LIST OF TABLES

		Page
Table 1	Data on Patients Testing Positive for *S. typhi* 'O' agglutinins (≥160)	69
Table 2	Biochemical Differentiation of *Salmonella* Serotypes	73
Table 3	Number and Percentage of *S. typhi* and *S. paratypi* isolates from stool and Urine samples	74
Table 4	Monthly frequency of Typhoid and Paratyphoid Fevers	75
Table 5	Age and Sex Distribution of Cases of Isolation of *S. typhi* and *S. paratyphi*	76
Table 6	Distribution of Cases of Typhoid and Paratyphoid Fevers according to Occupation in Zaria during the period of May to October, 1996	77
Table 7	Distribution of Cases of Typhoid and Paratyphoid Fevers according to Welfare Status	78
Table 8	Occurrence of symptoms among the 36 cases of Typhoid and Paratyphoid Fevers	79
Table 9	Results of Antimicrobial Susceptibility Testing of *S. typhi* Isolates	80
Table 10	Results of Minimum Inhibitory Concentration (MIC) Tests on *S. typhi* Isolates	81
Table 11	Minimum Inhibitory Concentrations (MIC) of Chloramphenicol, Ampicillin-Sulbactam and Trimethoprim - Sulfamethoxazole for the *S. typhi* isolates	82

LIST OF FIGURES

		Page
Figure 1	Monthly frequency of typhoid and paratyphoid fevers	83
Figure 2	Age and Sex Distribution of Cases of Typhoid and Paratyphoid fevers	84
Figure 3	Distribution of Cases of Typhoid and Paratyphoid fevers according to occupation	85
Figure 4	Distribution of cases of Typhoid and Paratyphoid fevers according to Welfare status	86

LIST OF APPENDICES

		Page
Appendix I	Data On The 500 Patients Examined, with Widal Test Results	108
Appendix II	Zone diameter Interpretive Standard for Antibiotic Sensitivity Test	120

CHAPTER ONE

INTRODUCTION

Typhoid fever is one of the most important infectious diseases throughout the world (Hashimoto et al., 1995). The infection is characterized by fever, malaise, headache, abdominal pain, diarrhoea and sometimes constipation, nausea and vomiting, anorexia, insomnia, exhaustion, apathy or mental confusion. A rash (rose spots) may be seen on light coloured skin. The fever lasts for three to four weeks, relapses are common and death may result from complications (Cheesbrough, 1985; Mackie & McCartney 1978; Olle-Goig et al., 1993).

Typhoid fever is caused by the bacterium Salmonella typhi belonging to the genus Salmonella. Two other members of this genus, namely Salmonella paratyphi A and Salmonella paratyphi B (S. schottmulleri) cause the paratyphoid fevers which together with typhoid fever, are generally referred to as enteric fevers (Jawetz et al., 1984).

The typhoid and paratyphoid pathogens are essentially human parasites and are acquired almost exclusively from human sources. They are excreted in the faeces and urine of patients and carriers and infection is mostly by ingestion of food or water contaminated with the organisms and sometimes by direct ingestion of the organisms from contaminated hands.

The incidence of typhoid fever is closely associated with the availability of safe water supply, food and personal

hygiene and environmental sanitation. In the United States, Japan and United Kingdom, where safe water supply is readily available and standards of environmental sanitation and food hygiene are high, typhoid fever has been virtually eliminated (Eykyn & Williams, 1987; Mackie & McCartney, 1978; Yew et al.,1993). However, the infection continues to pose an important public health problem in many developing countries in Africa, South America and Asia. It remains endemic in such areas without adequate sewage disposal or protected water supply and the highest incidence of typhoid fever occurs where a contaminated water supply serves a large population (Al Qurawi et al., 1995; Ivanoff et al., 1994; Klugman et al.,1987).

In Zaria, typhoid fever is reported to be common (Akoh, 1987; Bala & Auty, 1977). Situated at 11°N and F"40E, in the heart of Northern Nigeria, Zaria possesses a tropical continental climate. Rainy season starts from April or May, reaches its peak in August and ends in October. The urban area of Zaria is made up of four units - the old walled city (Zaria City), the colonial township (Sabon gari), Tudun Wada and the ABU - Samaru Village area. Farmers, traders, craftsmen and civil Servants form most of the population of Zaria (Hore, 1970;Norman, 1970;Urquhart, 1970).

Most people of low socio-economic status in Zaria live in areas characterized by overcrowding and poor sanitation. Pit toilets are common and drinking water is obtained from largely

unprotected wells which are flooded during the rains. Contamination of water from the wells by flooding as well as the possibility of pathogens reaching the wells from the pit toilets through the soil may be important factors in the transmission of typhoid fever in Zaria. In the dry season, the concentration of the organisms in the partially dry wells and rivers, increase in fly breeding close to homes and contamination of food and cutlery from the pit toilets by flies all serve to maintain the infection all year round and ensure its endemicity in Zaria. As most of the food venders and street hawkers belong to this socio-economic group, they contribute to poor food hygiene and environmental pollution (Akoh, 1987; Bala & Auty, 1977).

Typhoid fever persists as a serious public health problem in many developing countries (Ivanoff et al., 1994); Lanata et al., 1990). This situation is aggravated by the increasing incidence of antibiotic resistant strains which lead to the occurrence of more severe clinical disease and higher rates of morbidity and mortality (Thong et al., 1995; Bhutta et al., 1991; Holmberg et al.,1987). This problem is made more urgent by the movement of these resistant strains from one geographic location to another due to migration (Hoffman et al., 1984).

Therefore the present study was carried out in order to determine:

1. the prevalence of typhoid fever among hospital patients with febrile illnesses,

2. the distribution of cases by age, sex and socio-economic status,

3. the antibiotic sensitivity pattern of S. typhi isolated from clinical samples obtained from the patients.

4. The minimum inhibitory concentration (MIC) of the antibiotics for the S. typhi isolates.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Historical Review

Typhoid fever was for many centuries confused with a lot of other fevers especially typhus. The confusion between typhus and typhoid was not easily resolved and different authors credit different researchers with distinguishing the two syndromes. Ivanoff et al., (1994), gave the honour to C.A. Louis of Paris who was said to have described typhoid in 1929 and clearly separated it from other fevers, relating the clinical features to lesions in the intestines, mesenteric lymph nodes and spleen. Topley and Wilson (1964), mentioned that in 1839 Schoenlein recognized the two distinct diseases as 'tyhus exanthematicus' and 'typhus abdominalis'. Christie (1974), mentioned William Jenner who in 1850 conducted numerous detailed clinical and post-mortem examinations and clearly showed that the lesions in the Peyer's patches and the mesenteric glands were specific to typhoid and were never present in typhus. He also showed that the attack of typhoid fever did not give protection against the attack of typhus. According to Ivanoff et al. (1994), the infectious nature and transmission of typhoid fever was not known until Budd in 1873 provided evidence that bowel discharges were the main waterborne mechanism of infection. Eberth in 1880 discovered the etiologic agent in tissues from a patient suffering from typhoid fever and Gaffky in 1884 first cultivated and isolated

S. typhi in pure culture from the spleens of infected patients. Pfeiffer and Kolle (1896) in Germany, and Wright 1896 in England prepared the first vaccine for human use with heat-killed organisms. That same year Widal reported that convalescent-phase serum mixed with S. typhi led to the sticking together of organisms in clumps and losing their motility. The term "enteric fever" was introduced in 1869 in an attempt to focus attention on the anatomic seat of the constant primary lesion, although the intestinal lesion has since been shown not to be the primary lesion of typhoid fever.

2.2.1 The Genus Salmonella

The subcommittee on the Enterobacteriaceae of the International Committee on Nomenclature of Bacteria defines the genus thus:- The genus Salmonella consists of motile or non motile, nonsporing, gram-negative rods which may or may not have fimbriae, grow on ordinary media, reduce nitrates to nitrites, give a negative oxidase reaction and break down carbohydrates by a fermentative reaction that distinguishes them from other families in which the reaction is oxidative.

As described by Cruickshank et al., (1975), Salmonellae are gram-negative, non-acid fast, non-sporing and unencapsulated rods that belong to the family Enterobacteriaceae. They vary in length (2-4μmx0.6μm) and longer pleomorphic forms or short coccobacilli may develop after prolonged culture on laboratory media. All Salmonella

species are motile by means of peritrichous flagella except S. pullorum and S. gallinarum which are always non-motile.

2.2.2 Cultural Characteristics

The following cultural and biochemical characteristics of Salmonella are described by various authors. On nutrient agar, colonies are typically 2-4mm in diameter, grey-white, moist and circular with a smooth convex surface and entire margin. Their size and degree of opacity varies with the serotypes. For example S. paratyphi A form small colonies while S. paratyphi B form large mucoid colonies (Cruickshank et al., 1975). S. typhi, S. abortus-ovis, S. pullorum and S. choleraesuis grow less abundantly and produce small dew drop-like colonies even after 18-48h incubation (Buxton et al., 1977).

Salmonella are aerobes or facultative anaerobes that grow readily on simple media in the temperature range of 15-41°C. The optimal growth temperature is 37°C. They characteristically ferment glucose and mannose from which they form acid and sometimes gas but do not ferment lactose or sucrose (Jawetz et al., 1984).

Culturally, Salmonella are similar to most other enterobacteria but on differential media such as MacConkey agar or Deoxycholate citrate Agar (DCA), they form pale non-lactose fermenting colonies after incubation for 18-24h at 37°C, which are similar to those of the other common pathogenic genera Shigella. These are distinct from the pink,

lactose-fermenting coliforms (Mackie et al., 1978).

Plating media such as Salmonella-Shigella Agar (SSA) and Bismuth sulphite Agar (BSA) may be used for selective growth of Salmonella. On SSA, Salmonella appear colourless with black centers due to hydrogen sulphide production. Proteus species form similar colonies that are larger in size. BSA is highly selective for S. typhi. The colonies appear small, discrete and black with a distinct metallic sheen, giving the characteristic 'rabbit eye' appearance. Most lactose-fermenting coliforms and Shigellae are completely inhibited on BSA (Koneman et al., 1979).

2.2.3 Biochemical Properties

When stained by Gram's method, members of the genus Salmonella are indistinguishable from each other and from other members of the family Enterobacteriaceae. A series of biochemical tests are normally carried out to identify the different serotypes (Mackie et al., 1978). Typical Salmonella conform to the following patterns of biochemical reaction:-

All serotypes produce acid and gas from sugar fermentation except S. typhi and S. gallinarum which do not produce gas. Lactose, sucrose and salicin are not fermented (Skerman, 1967).

Salmonella decarboxylate the amino acids lysine, ornithine and arginine but S. typhi and S. gallinarum are exceptional in lacking ornithine decarboxylase (Edwards and Fife, 1961; Ewing, 1962).

Citrate is utilized by all members of the genus except S. typhi, S. paratyphi A, S. gallinarum and S. pullorum (Cruickshank et al., 1975; Ewing, 1986).

Urease production is used to differentiate the Salmonella group which are urease-negative from the urease-positive Proteus species.

Salmonella serotypes produce hydrogen sulphide gas and this property, together with motility, is used to distinguish them from Shigella species on sulphite-containing semisolid media (Koneman et al., 1979).

Salmonella are indole, Voges-Proskauer, ONPG and KCN-negative and methyl red positive. Nitrite is produced from nitrates, gelatin is not liquified and malonate and gluconate are not utilised. (Ewing et al., 1970).

TYPICAL BIOCHEMICAL REACTIONS OF SOME ENTEROBACTERIA

Test or Substrate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Hydrogen Sulphide (TSI aga)	-*	-	+	+	d	+"	+	d	-	-	-	-	+	_	_
Urease	-	-	-	-	-	-	-	d	+"or-	d"	-	d	+	+	+or(+)
Indole	+	-or+	+	-	-	-	-	-or+	-	-	-	d	+	-	+or+
Methyl - Red (MR)	+	+	+	+	+	+	+	+	-	-or+	-or+	+or-	+	-or+	+
Voges - Proskauer (VP)	-	-	-	-	-	-	-	-	+	+	+or-	-or+	-	+	-
Citrate (Simmons)	-	-	-	d	(+)*	-	+	+	+	+	d	d	d	+	-
K C N	-	-	-	-	-	-	-	+or-	+	+	+	+or-	+	+	-
Motility -37°c	+or-	-	+	+	+	+	+	+	+	+	+	+or-	+	-	+25°c
Gelatin (22°C)	-	-	-	-	-	-	(+)or+	-	(+)	+or(+)	-	+	+	-	-
Lysine decarboxylase	d	-	+	+	+	+	+	-	-	+	+	-	-	+	-
Arginine dyhydrolase	d	d	-	+or(+)	(+)	-or(+)	(+)or+	+or(+)	+	-	d	-	-	-	-
Ornithine decarboxylase	d	d	+	+	+	-	+	d	+	+	+	-	-	-	+
Phenylalaine deaminase	-	-	-	-	-	-	-	-	-	-	-	-	+	_	_
Glucose — Acid	+	+	+	+	+	+	+	+	+	d	+	+	+	+	+
Glucose — Gas	+	-	+	+or-	+	-	+	+	+	+or-	+	-or+	+or-	+	-
Lactose	+	d	-	-	-	-	d	d	+or(+)	-	d	d	-	+	-or(+)
Sucrose	d	d	-	-	-	-	-	d	+	+	d	+or-	+	+	+
Mannitol	+	+or-	-	+	+	+	+	+	+	+	+	+or-	-	+	+
Dulcitol	d	d	-	d	d	d	-	d	d	-	-	-	-	-or+	-

Salicin	d	-	-	-	-	-	-	d	+or(+)	+	d	d	d	+	d
Adonitol	-	-	-	-	-	-	-	-or+	-or+	-	-	-	-	d	-
Inositol	-	-	-	d	-	-	-	-	d	-	-	-	-	+	d 37°c
Sorbitol	d	d	-	+	+	+	+	+	+	+	-	-	-	+	+
Arabinose	+	d	d	+or(+)	-	-	+	+	+	-	+	+or-	-	+	+
Raffinose	d	d	-	-	-	-	-	d	+	-	-	d	-	+	-
Rhamnose	d	d	-	+	+	-	+	+	+or(+)	-	+	d	-	+	-
Malonate	-	-	-	-	-	-	+	-or+	+or-	-	+or-	-or+	-	+	_
Mucate	+	-	-	+or-	-	-	+or-	+	+or-	-	-	d	-	+	-
Jordan's D-tartrate	+	-or+	-	+or-	+or-	+	-	+or-	+or-	+	+or-	NT	+	+	+
Sodium acetate	+or(+)	-	-	d	-or(+*)	-	+or(+)	+or(+)	NT	+or-	NT	NT	d	NT	-
Maltose	+	d	+	+	+	+	+	+	+	+	+	-	+	+	+
Xylose	d	d	-	+	+	+	+	+	+	d	+	d	+or(+)	+	d
Trehalose	+	d	-	+	-	+	+	+	NT	+	+	NT	(+)or+	NT	+
Cellobiose	-	-	-	d	-	d	d	d	+	-	+or(+)	d	(+)or-	+	+
Glycerol	+or(+)	-	(+)or+	d	-	(+)	-or+	+	+or-	+	+	d	+or(+)	+	+or(+)
Esculin	d	-	-	-	-	-	-	-	-or+	d	-	+or-	d	+	d
Beta - galactosidase (ONPG)	+	-or+	-	-	-	-	+	+	+	+	+	NT	-	+	+
Nitrate to Nitrite	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

KEYS

\+ = 90% or more positive within 1 or 2 days

(+) = Positive reaction after 3 or more days

\- = 90% or more giving no reaction after 3 or more days

1. = Escherichia coli
2. = Shigella
3. = Edwardsiella

+or-	=	Most cultures positive, some strains negative
-or +	=	Most cultures negative, some strains positive
+or (+)	=	Most reactions occur within 1 or 2 days; some are delayed
*	=	symobols in parenthesis indicated occasional reactions
a	=	Occasional strains may produce H_2S in TSI
d	=	Different reactions, +, (+), -
W	=	Weakly positive reaction
NT	=	Not tested

4. = Salmonella subspecies 1
5. = Salmonella choleraesuis
6. = Salmonella typhi
7. = Arizona
8. = Citrobacter
9. = Enterobacter
10. = Serratia
11. = Hafnia
12. = Erwinia
13. = Proteus
14. = Klebsiella
15. = Yersinia

2.2.4 Classification

The Genus *Salmonella* belongs to the family *Enterobacteriaceae*. According to Ewing (1966), this family consists of four divisions and eleven genera, namely, *Escherichia* and *Shigella* (Division 1); *Edwardsiella*, *Salmonella*, *Arizona* and *Citrobacter* (Division II); *Klebsiella*, *Enterobacter* and *Serratia* (Division III); and *Proteus* and *Providencia* (Division IV).

The genus *Salmonella* contains a very large number of serotypes which are so closely related morphologically and biochemically. This has created some difficulties in naming. Moreover a minority of serotypes give biochemical reactions different from those just described as typical (Frobisher *et al.*, 1968).

Kauffmann (1969) divided the genus *Salmonella* into four subgenera: Subgenus I contains the majority of serotypes including all those that commonly cause disease in man and animals and give the typical reactions. The other subgenera - II, III, and IV give atypical reactions. For example subgenus III comprised of the *Arizona* group which some authors ascribe to a separate genus *Arizona*.

Another system of nomenclature which gained a wide acceptance was the Ewing's proposal. In this proposal, Ewing (1966), recognized three primary species of *Salmonella*,

S. typhi, containing only one serotype,

S. choleraesuis containing one serotype and

S. enteritidis, to which all other serotypes belong.

More recently, the name of the place where the strain was first isolated has been used. Scientifically, none of the present methods of nomenclature of Salmonella is satisfactory; however the Kauffmann-White scheme has been generally accepted for use in diagnosis. In this scheme each isolate is classified by antigenic analysis and the assignment of a specific name (Frobisher et al., 1968).

However, the taxonomy and nomenclature of Salmonella have changed over the years and are still evolving (McWhorter Murlin & Hickman Brenner, 1996). Studies on the relatedness of the deoxyribonucleic acids (DNAs) of bacterial species have added another dimension to the never ending attempt to classify Salmonella in a way that is acceptable to most taxonomists, and other investigations indicate that the DNAs of members of the three taxonomic units mentioned above are so closely related (70-100%) that they comprise a single relatedness group currently considered to be a subspecies rather than a species (Crosa et al., 1973; Le Minor et al., 1982a,b; Ewing, 1986). Moreover the entire group including typical Salmonella, members of the Arizona group and the atypical Salmonella (Subgenera II and IV of Kauffman) - are considered to be members of a single species - S. enterica, the DNAs of which are in the range of 61-100%. This species comprise of six subspecies of which two (3a and 3b) constitute the Arizona. The subspecies are (1) S. enterica subspecies

enterica (2) S. enterica subspecies salamae (3a) S. enterica subspecies arizonae (3b) S. enterica subspecies diarizonae (4) S. enterica subspecies houtenae (5) S. enterica subspecies bongori (Le Minor et al., 1982a,b Ewing, 1986).

On the basis of the foregoing, the following definition of the genus Salmonella was given by Ewing (1986): The genus Salmonella is composed of motile bacteria that conform to the definition of the family Enterobacteriaceae and the tribe Salmonellae. Hydrogen Sulphide is produced, the Methyl-Red reaction is positive, lysine and ornithine are decarboxylated, and arginine is dihydrolysed. Indole is not formed, and urea is not hydrolysed. The Voges-Proskauer test is negative and neither phenylalanine nor tryptophan is deaminated. Acid is not produced from sucrose, adonitol, raffinose or alpha-methyl-glucoside. Lactose is fermented by most strains belonging to subspecies IIIa and IIIb, but not by those of subspecies I, II, IV or V. Dulcitol is fermented by strains of subspecies I, II and V, but not by those of IIIa, IIIb or IV. Inositol is not fermented by cultures that belong to subspecies IIIa, IIIb, IV or V. The type species is Salmonella enterica subspecies enterica as exemplified by serotype Typhimurium.

Since this definition of the genus Salmonella was published by Ewing in 1986, subspecies VI has been described by Le Minor et al., (1986). Subspecies VI strains are inositol and sorbitol negative; 22% ferment lactose and 67%

ferment dulcitol. Le Minor and Popoff (1982) reported that subspecies V was distinct from the other subspecies of Salmonella and Reeves et al., (1989) proposed that subspecies V strains be placed in a second species which they designated S. bongori. Thus the genus Salmonella currently has two species:

S. enterica, comprising the following subspecies

S. enterica subsp. enterica (designated I or 1
S. enterica subsp. Salamae (" II or 2)
S. enterica subsp. arizonae (" IIIa or 3a)
S. enterica subsp. diarizonae (" IIIb or 3b)
S. enterica subsp. houtenae (" IV or 4)
S. enterica subsp. indica (" VI or 6)

S. bongori (formally subsp. V or 5).

"S. enterica" is not a valid species name. S. choleraesuis is the legitimate species name for strains in subsp. I - IV and VI but it can be confused with the common serotype that is also designated choleraesuis. The use of the species name "S. enterica" prevents confusion because there is no serotype attached to this name.

A majority of the Salmonella serotypes belong to subsp. I which include most serotypes in the first nine 0 groups (A, B, C_1, C_2, D, E_1, E_2, E_3, and E_4 as well as most strains isolated in clinical laboratories. Examples are S. enterica subsp enterica serotype Typhimurium, ser. Paratyphi A, Ser. Choleraesius, Ser. Typhi, Ser. Enteritidis, Ser. Gallinarum

and Ser. Pullorum. Subsp I are usually isolated from humans and warm-blooded animals. The biochemical reactions of this group are typical for Salmonella. They are dulcitol positive, tartrate-positive and galacturonate-negative, which distinguishes them from other subspecies. Moreover, citrate utilization is negative for S. typhi S. paratyphi A, S. gallinarum and S. pullorum. S. typhi and S. gallinarum do not produce gas from glucose and S. typhi alone cannot decarboxylate ornithine (McWhorter-Murlin & Hickman-Brenner. 1996).

2.2.5 Antigens of Salmonella

Salmonella contain several antigens. Important in the pathology of Salmonella are the somatic (0) antigens, flagellar (H) antigens and the capsular (Vi) antigen. Others are the fimbrial and K antigens.

The surface of the bacterial cell is composed of phospholipid-polysaccharide complexes which constitute the somatic (0) antigen. This antigen is hydrophilic, heat-stable at 100°C for 2.5 hours and alcohol-stable with 96% ethanol at 37°C for 4 hours. The former procedure destroys the flagellar and fimbrial antigen, while the latter detaches the flagellar. There is a large number of different 0-antigens occurring in the Salmonella group, each one specified by the nature of its terminal group and majority of the organisms possess more than one somatic antigens on their surfaces.

H-Antigens - Most Salmonella serotypes possess the flagellar

(H) antigen. This is a heat-labile, alcohol-labile protein complex which withstands 0.04-0.2% formaldehyde. There is a variety of these H-antigens distributed among the organisms and each one is specified by its amino acid content and the order in which they occur. Moreover, many organisms are capable of producing either one or two serologically distinct flagellar antigens classified as either phase I or phase 2 and are therefore referred to as either monophasic or diphasic (Kauffmann, 1975).

The K-Antigens, described by Kauffmann and Vahlne in 1963 are either envelope or capsular antigens and occur in many groups of enterobacteria. They are designated A,B, L, Vi, 5, M etc and have different biochemical properties. In the Salmonella group, three K-antigens, namely 5, Vi and M antigens have been demonstrated.

The 5-antigen was earlier designated as 0-antigen but was found to be more heat-labile and not connected with virulence.

The Vi-antigen occurs in S. typhi and S. paratyphi C strains. A few strains of S. dublin and Citrobacter freundii are also found to possess the Vi-antigen. It is present on the surfaces of newly isolated virulent strains but lost after repeated subculture. This antigen has been found to interfere with agglutination by 'O' antisera but this can be removed by heating the cell suspension since the Vi antigen is thermolabile. The discovery of this antigen by Felix and Pitt in 1934 greatly contributed to the elucidation of the serology

of S. typhi and solved the enigma of 'O' inagglutinability. The Vi-antigen contributes to the virulence of the bacteria since it protects the cells against phagocytosis and is also related to the chronic carriage state (Kauffman, 1975).

Mucoid forms of Salmonella possess the M (Mucoid) antigen. Kauffmann (1935) described the M-antigens of S. paratyphi B, S. potsdam, S. typhimurium and some other serotypes. The antigen causes 'O' and 'H' inagglutinability in mucoid forms which can be abolished by heating at 100°C (Kauffman, 1975).

The Fimbrial Antigen is probably a mutant form of fimbriae, being morphologically different from types 1 and 2 fimbriae of Salmonella. It also has practical importance in Salmonella diagnosis (Edwards and Ewing, 1972).

Salmonella 'O' antigens have been found in some strains of Escherichia, Shigella, Proteus and Citrobacter (Cruickshank et al., 1975).

2.2.5.1 The Kauffmann-White Scheme

This method of classification was initiated by White and Kauffmann in 1929 and finally published by Kauffman in 1977 (Buxton & Fraser, 1977). Presently, more than 2300 serotypes of Salmonella have been identified using this scheme (Pignato et al., 1995).

In this scheme each O antigen has been designated with an Arabic numeral 1,2,3---67 with a few numbers missing. Organisms possessing similar O antigens are placed in groups

A,B,C,D ---. The flagellar (H) antigens have two series of numbers depending on phase variations: Phase 1 antigens are identified by alphabets a -z (with j missing) and the remainder are given the letter z and a number, example z6. Phase 2 antigens are denoted by Arabic numerals which are not meant to represent those of the O antigens.

To write the antigenic formula of an organism each antigen is separated from the other by a comma. A colon separates the O antigens from the H antigen and a semicolon separates the phase 1 from the phase 2 antigens. Incomplete antigens are enclosed in brackets while a dash substitutes for an absent phase of H antigen. Example, S. typhimurium belongs to Group B and its antigenic formula is 1,4,5,12:i;1,2 which means that it possesses the somatic antigens 1,4,5 and 12, phase 1 (H) antigen i and phase 2 (H) antigen 1 and 2. S. typhi belongs to Group D1 and its antigenic formula is 9,12,Vi:d; - (Buxton & Fraser, 1977; Frobisher et al., 1968).

The Kauffmann - White scheme is updated annually. The current number of serotypes in each species and subsp is as follows:

S. enterica	2321
Subspecies I	1379
Subspecies II	466
Subspecies IIIa	93
Subspecies IIIb	309
Subspecies IV	64

Subspecies	VI	10
S. bongori	(V)	18
Total		2339.

The serotype name is written in Roman (not italicized) letters with the first letter in capital, example Salmonella serotype (abbreviated ser.) Typhimurium and S. Ser. Dublin. Unnamed serotypes are designated by their antigenic formulas following the subspecies name, example S. enterica subsp. diarizonae ser. 60:K:Z (abbreviated S. serotype 60:K:Z) (McWhorter-Murlin & Hickman - Brenner, 1996).

2.2.5.2 Antigenic Variation

Kauffmann (1957) distinguished between the following kinds of antigenic variation.

H-O variation: This is the loss of flagellar (H) antigens and transition of OH forms to O forms. This change is rare and irreversible. Certain serotypes such as S. pullorum and S. gallinarum only exist in the O form.

S-T-R variation: This is the loss of O antigens and transition of S (Smooth) forms to T or R (rough) forms. T forms are transient between the S and R forms. They contain a new thermostable antigen different from the O antigens but are morphologically indistinguishable from the S forms.

Form variation: This represents quantitative changes in somatic antigens and involves changes in O and Vi antigens in which they are either well-developed, poorly developed or

absent.

Phase variation or H variation: This is a qualitative change in the flagella antigen, each strain varying spontaneously and reversibly between two phases. Three kinds of phase variation are recognised:-

Specific - non specific phase variation described by Andrewes e.g b:1,2

α -ß phase variation of Kauffmann and Mitsui (1963)

e.g. b: e, n, x

Phase variation of Edwards and Bruner (1938) e.g. b:1,W Phase 1 include the specific and α phases while phase 2 covers Andrewes group or non-specific phases and ß Phases.

By infection with bacteriophages any of the variations described above can be transferred from one serotype to another by transduction (Kauffmann, 1957).

2.2.5.3 Serological typing

Serological typing of isolates is based on the use of adsorbed monospecific agglutinating sera, each representing a single different antigenic component of the various serotypes of Salmonella. With these one may test any organism for the presence of different H and O antigenic components and assign to it a "formula" expressing the antigenic complex of which it is composed. Serological typing is done in three stages. In stage 1 only a few antisera are required. These are (i) a polyvalent antiserum, (ii) O antisera for serogroups A to E (iii) antisera for flagellar antigens a,b,c and d and the 1 --

- complex (1,2;1,5;1,6 and 1,7 antisera) (iv) a single factor H antisera for factors 2 and 5 and (v) a Vi antiserum. (This stage may be modified by the deletion or addition of some of the antisera). These antisera permit complete characterization of several important Salmonella and partial characterization of many others. Stage 2 is intermediate. Fifty or sixty commonly occurring serotypes belonging to serogroups A-H,K,L and N, including serotypes Paratyphi A, Choleraesuis and Typhi are identified completely. Other antisera may be added for complete serotyping of particular strains that may become epidemic or endemic in an area. Stage 3 is complete serologic identification of all serotypes of Salmonella, including characterization of new serotypes. This work is restricted to a relatively small number of laboratories which are equipped for the work and which may serve as reference centers (Ewing, 1986).

2.3 Epidemiology

2.3.1 Distribution

Salmonella are widely distributed in nature and are associated with both wild and domesticated animal hosts, with poultry as the major reservoir (Swamy et al., 1996). Members of the genus Salmonella are normally found in the intestines of animals especially pigs, cattle, goats, sheep and poultry, from which man is sometimes infected. The most common source of Salmonella infections for humans are contaminated foods such as eggs, poultry, meat and meat products. A number of

Salmonella serotypes may be transferred to poultry from various sources such as feedstuffs, rodents and wild birds. Salmonella are not killed by drying and therefore survive in products such as dried egg or bone meal fertilizers (Casanovas, 1995; Cheesbrough, 1985,).

The genus Salmonella contains more than 2,300 different serovars. All these serovars are primarily pathogenic for animals but only a relatively restricted number belonging mainly to the species S. enterica subsp. enterica are responsible for the majority of human infections (Pignato et al., 1995).

The degree of host adaptation in Salmonella varies and affects its pathogenicity in man in the following ways:

1. Ubiquitous serovars such as S. typhimurium and S. enteritidis which affect both man and a range of animals cause gastrointestinal infections of varying severity (but usually less severe than enteric fever). In addition to classical food poisoning, these serovars are involved in infantile and travellers diarrhoea (NRC, 1985).
2. Serovars which are highly adapted to an animal host such as S. choleraesuis which has the pig as the primary host (causing swine paratyphoid), also causes a severe systemic illness in man. S. abortus-ovis and S. abortus-equi cause abortion in sheep and horses respectively. S. gallinarum and S. pullorum cause fowl typhoid and

pullorum disease respectively. These however usually produce no or very mild symptoms in man (Buxton & Fraser 1977).

3. Serovars adapted to man such as S. typhi, S. paratyphi and S. sendai (NRC, 1985). In humans Salmonella serovars produce a range of disease states from mild gastroenteritis to typhoid and paratyphoid fevers and potentially fatal septicaemia (Gianella, 1991).

The typhoid and paratyphoid bacilli are spread from the faeces and urine of a patient or carrier to other persons, directly or indirectly by the faecal-oral routes. In direct transmission, there is direct contact of the organisms with the hands of the new victim, the fingers conveying the bacilli to the mouth. This happens frequently in persons caring for patients or debilitated carriers especially if the infection is not recognized and special precautions not observed. Indirect transmission occurs through consumption of water and food contaminated with the organisms or contamination of food and cutlery by flies carrying the bacilli on their feet or mouth parts. Fomites are involved less frequently and usually if there is direct contact (Anderson et al. 1969). Indirect transmission is more important because it is responsible for community spread of the disease (Christie, 1974).

2.3.2 Salmonella typhi and the environment

Water: The typhoid bacilli do not multiply in water and survives only for a few weeks. Results of studies on the

purification of water supplies show that over 90 percent of these organisms die out after three to four weeks holding. Factors such as temperature, presence of dissolved oxygen and organic matter favour the survival of typhoid and paratyphoid organisms and so these die more rapidly in a reservoir than more open or contaminated waters. The possibility therefore of organic matter or a carrier contaminating the water at a late stage of storage is an obvious danger (Christie, 1974). These reports are similar to that of Al Qurawi *et al.*, (1995) who reported rapid multiplication of *S. typhi* in water containing small amounts of organic matter.

Salmonella organisms may survive in sewage for several weeks though they are subject to competition with other organisms which may outgrow them. In one investigation, *S. typhi* survived for 38 days in a specimen of sewage kept at room temperature and *S. paratyphi* B for 21 days. Under natural conditions, sewage is likely to be continually replenished with the organisms if there are carriers or cases in the area (Christie, 1974).

Sewage treatment plants are designed to purify wastewaters for given parameters but their microbiological effectiveness is often poor without addition of a specific treatment such as disinfectant, filtration etc. Large amounts of enteric bacteria are thus released into the environment, in rivers and seawater. Whether subsequent survival in these

hostile environments is influenced by their previous stay in wastewaters remains a subject of research. Studies by Dupray & Derrien, (1994) showed that Enterobacteriaceae could adapt to sea water stress during their stay in sewage and sewage treatment plants. A previous stay in sewage could preserve or even induce some enzymatic activity which was then maintained during exposition to sea water. This suggests an active metabolism enabling new protein synthesis.

Sea water is bactericidal to S. typhi (Nabbut & Kurayiyya, 1972), but in coastal towns, sewage is usually discharged into the sea while still crude and untreated and Salmonella can be isolated regularly from such water exposed to sewage contamination.

Food: Shellfish is a common source of infection with S. typhi and S. paratyphi. Oysters and mussels may filter as much as ten gallons of sea water daily, thereby concentrating microorganisms inside their bodies. If they are lying in typhoid polluted water, they may harbour large numbers of the organisms. In view of this, there is always the risk of infection associated with eating raw shellfish which have been caught in contaminated sea shores or river estuaries. Experimentally, S. typhi can survive in infected shellfish for as long as four weeks (Christie, 1974). Shellfish is a particularly dangerous source of infection as it is normally eaten raw. Rippey (1994) reported 3770 typhoid cases in USA between 1988 and 1990 associated with consumption of raw or

partially cooked shellfish.

At a favourable temperature, S. typhi and S. paratyphi multiply freely in milk, cream and other foods that are not too acidic. They may live for over a month in butter and cheese. In carbonated beverages, they survive for about a week (Anderson et al., 1969).

As reported by Anderson et al., (1969), baked or deep fried foods may be potential vehicles of infection as food is a very poor conductor of heat and temperatures that are high enough to cook or even burn food at the surface may only serve to incubate bacteria in the interior.

Desiccated coconut sometimes contain Salmonellae of various types including S. paratyphi B. The number of Salmonellae in a sample of desiccated coconut is usually small due to its low moisture content, but when the coconut is added to cakes or creams, conditions are greatly improved for the Salmonellae and they multiply rapidly (Herbert, 1965).

Infection with S. typhi and S. paratyphi from meat is not common, but when the opportunity is offered, S. typhi and S. paratyphi B can both flourish in meat. Both cooked and cold meats may be contaminated by a carrier handling it after cooking or processing. Canned food is in general safe because the temperature reached in the sterilizing process is sufficient to kill all non-sporing pathogens but S. typhi introduced into cans of corned beef have been shown to multiply rapidly (Lyne & Tomlinson 1964).

2.3.3 Sources of Infection during outbreaks of typhoid fever

During the last century water has been the principle vehicle of infection with organisms causing enteric fever in the developed countries such as United States, Britain, Canada and Japan. According to Topley & Wilson (1964) it was responsible for tremendous outbreaks and widespread prevalence of typhoid.

With the recognition of the spread of disease through water and development of methods of water purification by Budd in 1874 and Snow in 1855, there was a remarkable decline in the incidence of typhoid fever.

Presently, food is the principal vehicle in developed countries and is most commonly contaminated by an unrecognized case or carrier who is employed as a food handler or from hands accidentally soiled at the time of defecation (Ivanoff et al., 1994). Outbreaks of typhoid and paratyphoid fevers have been caused by consumption of milk and cream contaminated with the organisms and these tend to be explosive in onset and numbers due to the large number of the organisms likely to be present in these products (Christie, 1974). Outbreaks of infection in man have been traced to the consumption of cooked or uncooked coconut (Herbert, 1965). Most of the outbreaks due to meat have been caused by cold meats contaminated by handling. Cans of corned beef contaminated by S. typhi sucked in with cooling water may cause a food borne outbreak

(Bashford & Herbert, 1965).

Contrarily, consumption of contaminated water is still the chief mechanism of infection in developing countries where raw river water and unprotected water wells remain widespread as source of drinking water. Thus water-borne transmission remains an important factor in disease epidemiology and because water bodies serve a large number of the population, the outbreaks tend to be explosive (Jamieson et al., 1972).

2.3.4 Typing Methods in Epidemiological Analysis of S. typhi Infection

It is very important in epidemiological studies of an infectious outbreak to trace the probable origin of the infection. This is made possible by distinguishing the particular strain of the pathogen causing the infection or epidemic.

Phage typing has been in use as a method of differentiating strains of organisms on the basis of differing susceptibilities to type-specific bacteriophages. Phage typing of Salmonellae was first applied to S. typhi by Cragie and Yen in 1938. It has since been adapted to a number of other serotypes (Frobisher et al., 1968). Phage typing may allow subdivision of a serological entity as in the case of serotype S. typhi which is divisible into eighty different phage types (Mitruka, 1976).

In recent years the value of molecular methods for discriminating between strains of pathogenic bacteria in defined epidemiological settings has been well proven in

numerous studies (Altwegg et al. 1989).

Ribotyping has been applied by Altwegg et al. (1989), who reported that ribosomal RNA gene restriction pattern (ribotyping) analysis was able to discriminate S. typhi strains, indicating considerable genetic diversity.

Analysis of envelope protein profiles of sixty-nine S. typhi strains by Franco et al., (1992) showed that there are genotypic and phenotypic differences among S. typhi strains which, they concluded, resulted in marked differences in the clinical manifestations of typhoid fever in different parts of the world.

2.3.5 Susceptibilities of Salmonella to Physical and Chemical agents

Salmonella are relatively susceptible to physical and chemical agents. A temperature of 55°C for 1 hour or 60°C for 20min is lethal to these organisms; and they are killed rapidly by autoclaving at 120°C. The lethal effect of heat on the organism in human foods varies according to the serotypes involved and the material which is contaminated. As a general rule, milk should be heated to 65.0°C for 1-2 secs., egg yolk to 60°C for 30 secs and meat to 65°C for 10-15 minutes (Christie, 1974).

Salmonella have a high susceptibility to phenol, cresol and formaldehyde vapour which is used to fumigate equipment in poultry farms, but the efficiency of these agents against the

organisms is reduced in the presence of faeces, colloidal or other organic matter (Buxton & Fraser, 1977). On the other hand Salmonella are resistant to brilliant green, sodium tetrathionate and sodium deoxycholate which all inhibit coliforms and are therefore useful for isolation of Salmonellae from faeces (Jawetz et al., 1984).

2.3.6.1 Incidence of Typhoid Fever

Typhoid fever is a worldwide disease. It is found in all countries and climates. In developed countries, its incidence has been reduced to well below 1 per 100,000 population and majority of the cases are imported from other countries by travellers (Jawetz et al. 1984; Yew et al, 1993). It remains a major public health problem in many developing countries, with an annual incidence of 16-17 million cases and approximately 600,000 deaths (Thong et al., 1995). Attack rates as high as 1100 cases per 100,000 population have been documented (Ivanoff et al., 1994).

Typhoid fever has been found to be highly prevalent in rural Haiti where the hospital reported cases number up to 74 per 100,000 inhabitants (Olle - Goig & Ruiz, 1993). The disease is endemic throughout the middle East. For example in Indonesia, it has a mean of 600,000 cases per year and more than 20,000 deaths (Ivanoff et al., 1994).

Typhoid fever is endemic throughout Africa. Its high prevalence has been reported from Ghana, Uganda and Nigeria (Hunter et al.,1966). It has been estimated that in Africa

with a population of $531x10^6$, the incidence of typhoid fever is 500 per 100,000 per year (Ivanoff et al., 1994).

2.3.6.2 Seasonal Variation

The incidence of typhoid fever varies with the season of the year. According to the report of Akoh (1987), cases of typhoid fever occurred throughout the year in Zaria, Nigeria but with a definite clustering of cases during the rainy season (May - Oct.). He reported two peaks - one coinciding with the onset of rains in May and the other at the height of rains in August. Archampong in Ghana (1969), reported a seasonal incidence of two peaks coinciding with the two rainy seasons in Accra. In Lagos, three peaks were reported - April, July and November but the disease appears to be present all the year round (Ogunbiyi & Onabowale 1976).

2.3..6.3 Age and Sex Incidence

No age is exempt from typhoid fever attacks. However, typhoid fever is regarded by most authors as a disease of the older child and young adult. Bala & Auty, (1976), in a study of typhoid patients, showed that 64% of the cases were under fifteen years with a mean age of nine years while Akoh (1987), noted a peak age group incidence of 5-9 years followed closely by 10-14 year. He observed that the prevalence of typhoid fever in persons over 30 years was low in Zaria. Okolocha et al., (1993) also noted a peak age group incidence in the 0-10 year group.

Contrarily, Ogunbiyi & Onabowale et al., (1976),

reported that the peak age incidence was in the second and third decades of life. Also, Anderson et al., (1969), noted that the highest incidence was in the late teens and early twenties and that cases were rare in persons over sixty. Al Qurawi et al., (1995), showed that all age groups were affected, but noted the absence of patients under one year of age, which was attributed to the protected life style and role of breast milk in that age. A low incidence among adults over thirty was attributed to acquired immunity but the apparent rarity of typhoid fever in children under five years of age was attributed to occurrence of mild clinical forms of the disease. In agreement with these reports, Ferrecio et al., (1984) noted in the endemic areas of S. America that the age specific incidence was low in under three year olds, high, with a peak among school children aged five to nineteen years and low in adults over thirty-five years. From the results of a study based on single blood culture from children under twenty-four month of age it was observed that 3.5 percent had an unrecognized bacteremia due to S. typhi or S. paratyphi which was not suspected on clinical grounds.

Reports from most authors agree that males are slightly more frequently infected than females. Male: female attack rate ratios of 2:1 (Al Qurawi et al., 1995), 1.13:1 (Akoh, 1987), and 1.07:1 (Okolocha et al., 1993) have been reported.

2.3.6.4 Socio-economic Factors

Typhoid fever is reported to be more prevalent among

people of low socioeconomic status than those of high socioeconomic status. Akoh (1987) reported a low to high socioeconomic status ratio of 67.19% to 10.95% in the prevalence of typhoid fever. This he attributed to the better housing and sanitation enjoyed by most people of high socio-economic class, most of whom have access to good potable water and modern toilet facilities. For most people of low socio-economic status the reverse is the case and most of them obtain drinking water from sewage polluted rivers and unprotected wells and use pit toilets, while some do not have toilets in their homes (Idoko et al., 1985).

2.4.1 Pathogenesis

Infection by S. typhi follows ingestion of the organisms. Various authors agree that the mean infective dose of S. typhi is 10^5-10^9, but doses as low as 10^3 have been quoted as being sufficient to cause disease (Glynn et al., 1995; Ivanoff et al., 1994). The incubation period varies greatly depending on the vehicle of infection. It is longest in water borne infection due to the few number of organisms likely to be present, and shortest in food-borne infection. The incubation period usually is 10-14 days (Christie, 1974).

The ingested Salmonellae reach the small intestine and enter lymphatics that carry them to the blood steam. They are then carried by the blood to many organs especially the spleen, gall bladder and intestine where they multiply in

lymphoid tissue. Very rarely S. typhi may be isolated from stool or blood during this period of incubation (Christie, 1974). This primary bacteremia takes place in the first 7-10 days of the incubation period.

After multiplication in the organs, bacilli pass into the blood, causing a secondary and heavier bacteremia, the onset of which coincides with that of the pyrexia (fever) and other signs of clinical illness (Mackie & McCartney, 1978).

The essential lesion in typhoid and paratyphoid fevers is caused by a proliferation of endothelial cells of the reticuloendothelial system. The accumulation of cells encroach on the local blood vessels and eventually produce inflammatory foci in many organs and in some areas, necrosis and sloughing of tissues. These occur most commonly in the lymphoid tissue of the intestinal wall, the mesenteric lymph nodes and the spleen. The most characteristic lesion produced is the ulceration of the intestinal mucosa at the Peyer's patches of the small intestine, sometimes with hemorrhage or perforation.

As a result of infection, the spleen may enlarge and then its palpation provides a valuable aid to clinical diagnosis. The organisms may settle in the spleen from where they may emerge to cause clinical relapse or theoretically to perpetuate the carrier state.

The liver is also involved, often with enlargement. The organisms infect the gall bladder through the bile duct and

multiply in the bile, a process facilitated by the presence of gallstones. The gallbladder is the main source of infection of the intestinal contents and often involved in the establishment of the chronic carrier state (Christie, 1974).

The role of endotoxins in the pathogenesis of typhoid fever has been studied in detail, but the results are not conclusive (Freisman et al., 1969). When volunteers were given an intravenous injection of endotoxin preparation from S. typhi, they developed symptoms very similar to those produced by infection of typhoid fever. Results of experiments with rabbit models suggest that the areas of focal necrosis and hemorrhage may be due to altered reactivity to catecholamines induced by endotoxins (Hornick et al., 1970).

Salmonella cause disease by invading the intestinal epithelium after ingestion (Finlay & Falkow, 1989). Researchers have studied the internationalization of Salmonella such as S. typhi, S. typhimurium and S. choleraesuis in epithelial cells and found that they multiply within formed vacuoles (Gahring et al.,1970). The intracellular infection of S. typhi has been previously reported by Christie (1974) who noted that the organisms are very scanty in the extracellular spaces but are mainly seen within the phagocytes and other tissue cells of the host and when inside the phagocytes are protected against the effect of antiserum. Studies by other authors also indicate that inside the host tissue cells, S. typhi may be protected against the

full lethal effects of some antibiotics unless the course of antibiotic treatment is long enough to destroy the host cell (Hopps et al., 1961; Showacre et al., 1961).

2.4.2 Clinical Presentation

Typhoid fever is a typical febrile septicaemic infection. The onset is normally insidious with malaise as a typical presenting symptom. In most cases there is continued fever (39°-40°C), anorexia, myalgia (muscle pain) and joint pains, abdominal pain, persistent headaches and insomnia. Sorethroat and a bronchitic cough are common in the early stages of the disease. Other symptoms of the infection include bradycardia (slow pulse rate), toxaema, splenomegaly and hepatomegaly (enlargement of the spleen and liver respectively), hyperplasia (excessive formation of cells) and lymphadenopathy. During the period of fever, about 25 percent of light skin-coloured patients show exanthema (rose spots) on the trunk. There is leukocytopenia with leukocyte count often below 4500/mm^3. Constipation is typical in older children and adults while diarrhoea may occur in younger children. A few individuals may develop coated tongues. Exhaustion persists throughout the illness and for a varying length of time after disappearance of symptoms (Bhutta, 1996; Christie, 1974; Ivanoff et al., 1994).

The fever lasts for three to four weeks. The organisms appear in the blood during the first two weeks of infection and in faeces and urine after the second week. The course of

the disease is prolonged and relapses are common (Drutz & Graybill, 1976).

The typhoid disease may vary widely. Mild forms have been known to occur especially in children. Sometimes the onset is abrupt and the symptoms may resemble those of streptococcal or pneumococcal septicaemia. If joint pains are severe, the illness may resemble the early stages of rheumatic fever. Headache, which may be severe enough to simulate meningitis may dominate other symptoms (Christie, 1974).

2.4.3 Complications

Typhoid fever has many complications. The most frequent are relapse, hemorrhage and perforation.

Relapse is manifested as a return of fever and other clinical symptoms of the disease, with the isolation of the original Salmonella type from blood, eight to nine days after the patient first become asymptomatic (Svenungsson, 1982). However, periods as short as one day and as long as seventy days have been noted. The relapse usually lasts for about eleven days but may continue for up to four or five weeks. Relapses occur in 10 to 20 percent of typhoid cases (Gadehold & Madsen, 1963). It has been observed that antibodies do not prevent relapse and that in chloramphenicol-treated patients relapses often occur when the antibody titre is highest (Watson, 1957).

In intestinal hemorrhage complication, there is passage of blood from the bowel by the middle of the second week of

the disease, due to bleeding caused by ulceration at the Peyer's patches. Hemorrhage may be mild, or severe and fatal. Its incidence varies and may depend on the social and nutritional state of the population being studied but is rare in children. Chloramphenicol therapy has greatly reduced the incidence of intestinal hemorrhage (Huckstep, 1962; Rowland, 1961).

Intestinal perforation is the most serious complication of typhoid fever and often requires surgical management (Badejo & Arigbabu, 1980). In the pre-antibiotic era, mortality associated with perforation approached 100 percent. Chloramphenicol therapy has reduced the associated mortality, but not the frequency of perforations (Woodward & Smadel, 1964). According to some authors, the incidence of perforations vary between 0.7 to 4.7 percent (Marmion, 1952; Rowland, 1961). In Zaria, perforation has been reported to be the commonest complication, accounting for 54 percent of typhoid fever deaths (Akoh, 1987).

Intestinal perforation presents the typical syndrome of sudden acute abdominal pain with swollen and tender abdomen, which may persist for days. In its most serious form, there is flooding of the peritoneal cavity with intestinal contents, vomiting and signs of collapse. Appendicitis may occur as a rare complication (Christie, 1974).

There are several other complications of typhoid fever, many of which are rare. These include:

- Pulmonary symptoms (Svenungsson, 1982),
- Cardiovascular complications, for example myocarditis (Gadehold & Madsen, 1963).
- Genito-urinary complications: Asymptomatic bacilluria is common in the early stages. Nephritis and pyelonephritis also occur rarely (Mathai et al., 1995).
- Neurological complications may occur in a small percentage of patients and could be severe with mental confusion, delirium and coma. Less commonly meningitis may occur (Osuntokun et al., 1972).
- Bone and joint complications, manifested mainly as pains commonly referred to as typhoid arthritis. This form may be accompanied by abscess formation from which S. typhi is discharged (Christie, 1974).

In the pre-antibiotic era, the mortality rate in typhoid fever was about 10 percent and death was usually as a result of hemorrhage or perforation through a necrotic Peyer's patch. However, presently available antimicrobial therapy has reduced the mortality rate to about 2 percent (Anderson et al., 1969, Bhutta, 1996). Nevertheless, data from developing countries paint a very different picture with case fatality rates ranging from 2 to 33 percent (Butler et al., 1991; Ellis et al., 1990), for instance there have been reports of about 21 percent case fatality in Zaria (Akoh, 1987), 20.3 percent rate in Lagos (Ogunbiyi & Onabowale, 1976) and 24 percent rate in Ibadan (Ikeme & Anan, 1966).

2.4.4 Carrier Rate

After recovery from typhoid and paratyphoid fevers, most individuals continue to harbour and excrete the organisms for variable lengths of time. These are either convalescent or chronic carriers. The convalescent carrier state ends in less than one year after infection, usually, by the end of the third month. However, about 2 to 3 percent of survivors from typhoid fever continue to be carriers for the remainder of their lives and excrete the bacilli in their faeces and urine. The site of growth of the organisms in the chronic carrier is usually the gallbladder, (accounting for about 90-95 percent of all carriers), the intestine and the kidney. It is rare for the chronic carrier state to cease without surgical removal of the site of infection (Anderson et al., 1969).

In developing countries, chronic carriers among commercial food handlers play a prominent role in the transmission and endemicity of the disease (Ivanaff et al., 1994). In general, the prevalence of chronic S. typhi carriers is greater among women, increases with age and in cases of gallbladder involvement, relates to the presence of chronic calculous cholecystitis. In endemic areas, the prevalence of chronic S. typhi carriers may range from 500 to 700 per 100,000 inhabitants (Lanata et al., 1990).

2.5 Diagnosis

Enteric fever can be difficult to differentiate from other causes of fever without access to bacteriological

culture facilities (Ross & Abraham, 1987). Even so, the conventional culture method for detecting S. typhi in clinical samples takes 2 to 5 days; and sometimes more than a week with blood samples (Hashimoto et al., 1995). Moreover, culture facilities are severely limited in many developing countries, hence the diagnosis of typhoid fever has always proved difficult and a single Widal test continues to be used extensively as a diagnostic tool (Buck et al., 1987).

The classic method used in the Widal test is the tube agglutination technique employing serial dilution of patients' sera, but a rapid slide agglutination method developed by Welch and Mickle in 1936 is used as an initial screening test to detect the presence of antibodies in patients' sera (Freter, 1980; Raphael & Spencer, 1983).

The value of the Widal test in the diagnosis of typhoid fever is controversial and different investigators employ different titres as criteria for positive diagnosis. Some report that an anti 'O' titre of ≥ 160 for S. typhi or a four-fold antibody increase is necessary for diagnosis (Raphael & Spencer 1983). Usually an anti - 'O' antibody titre of ≥ 80 or a two-fold rise in febrile agglutinins is regarded as diagnostic by most authors (Bala & Auty, 1977; Buck et al., 1987; Nichols & Nakamura, 1980).

In about 80 percent of carriers, Vi agglutinins persist in a titre of more than 5 and have been found useful in identifying the carrier state.

The interpretation of the Widal reaction may be obscured by various factors including a history of previous infection, the possibility of previous subclinical infection and a history of immunization. All these give rise to background titres in a population, thus titres which can be considered significant vary with the endemicity of the infection and no definite criteria can be stated (Sussman, 1975).

The Widal test is normally confirmed by bacteriological culture of patients' blood, stool, urine or bone marrow samples to isolate S. typhi or S. paratyphi (Cruickshank et al., 1975; Gilman, 1975).

It is vital to the management of Salmonella infections that the pathogen is isolated. In order to achieve this aim, numerous plating media have been described. Traditional media include a range of brilliant green agars, several deoxycholate - citrate based agars, MacConkey agar, Salmonella - Shigella (SS) agar and Bismuth Sulphite agar (BSA) (Macfaddin, 1985; Fricker, 1987). New media developed include Rambach agar, Novobiocin brilliant green glycerol lactose agar and Lysine Mannitol glycerol agar (LMG) (Stallard & Cox, 1994).

The organism may be recovered from blood during the first 7 to 10 days of clinical illness and during relapses, and as reported by Watson in 1967, S. typhi has been isolated from the blood in patients with neither symptoms nor history of typhoid fever.

Five to 10ml of venous blood should be cultured in an

appropriate broth such as Thioglycollate broth. This is subcultured into a fresh broth after 2 days. A second subculture may be necessary, then a loopful is streaked onto blood agar and MacConkey agar (Mackie & McCartney 1978). However, as reported by Ross & Abraham (1987) and Watson, 1957, more than half the cases can be blood culture negative because of few number of organisms in the blood (which may be as few as one organism per ml), and depending on the stage of the infection. According to Topley & Wilson (1964), absence of growth in broth should not be readily accepted as proof of absence of organisms from the blood stream and cultures should not be discarded for 10 to 12 days. It has also been noted that blood contains substances which are bactericidal to enteric organisms and so it is essential that blood must be diluted at least four times by the culture medium in order to reduce its bactericidal properties. (Topley & Wilson 1964; Watson, 1957).

Alternatively, clot culture may be performed by collecting the blood in a sterile universal container and allowing it to clot. The serum is then removed to perform a Widal test and the clotted blood cultured in streptokinase broth. The role of streptokinase is to lyse the clot while also greatly reducing the bactericidal activity of the clot. Watson (1957), recommended that the clot should be added to bile salt broth containing 100 units streptokinase per ml. The advantages of clot culture over blood culture is that it

may yield a higher percentage of positive results since the organisms are concentrated in the clot (Cruickshank et al., 1975).

Specimens of faeces and urine should also be cultured in an endeavour to isolate the S. typhi. If stool or urine culture yields S. typhi in the absence of a compatible clinical illness or high antibody titre, the patient may be a carrier. Traditionally, stool cultures are usually positive from the second week and urine cultures from the third week of infection (Mackie & McCartney 1978), but with modern bacteriological methods, in at least 50 percent of cases, culture of the faeces is positive in the first week with the number of organisms per gram of faeces increasing greatly in the second and third weeks of untreated disease. According to Christie (1977), a negative report does not rule out diagnosis of typhoid fever because there are technical difficulties in separating the enteric group of organisms from other organisms that inhabit the intestinal tract. Recovery of S. typhi from urine is a rare event even in areas endemic for typhoid fever (Mathai et al., 1995).

Stool and urine specimens are inoculated into enrichment media for example tetrathionate or selenite broth and after incubation at 37°C for 18 to 24 hours, the growth is subcultured to selective and differential plating media such as mentioned above. Colonies which give cultural characteristics similar to Salmonella species are subjected to

biochemical tests.

2.6 Therapy of typhoid fever

With the introduction of chloramphenicol in 1948, effective therapy in the enteric fevers became available (Mackie & McCartney 1978). Chloramphenicol is still the recommended drug of choice in many countries (Akoh, 1987; Eykyn & Williams 1987; Gulati et al. 1992; Raghupathy et al. 1992). However, there are limitations in the use of chloramphenicol. Even though the drug exerts a marked bacteriostatic effect against S. typhi, it has little effect on the intracellular pathogen (Christie, 1974). For effective therapy, chloramphenicol has to be used in large doses for a long period. A great deal of experimentation on timing and dosage have been carried out in order to reach a favourable conclusion. Christie, (1964) recommended an adult dose of 500mg every four hours till temperature has settled, usually in 3 to 5 days and thereafter, 500mg every six hours until the fourteenth day. For children, 50mg per kg body weight per day is recommended, reducing to 25mg after the temperature is normal. More recently a children's dose of 50 to 100mg per kg body weight per 24 hours has been given (Raghupathy et al., 1992).

The problem of long course of treatment is compounded by the fact that chloramphenicol can produce serious side effects, the most serious being bone marrow suppression and ensuring blood dyscrasias (aplastic anemia, hypoplastic

anemia, thrombocytopenia and granulocytopenia) developing weeks or months after oral therapy. The likelihood of such disorders developing and proving fatal in people receiving chloramphenicol is significant with a fatality of one per 25,000 to 40,000 patients receiving chloramphenicol (Molina, 1987).

Because of the awareness of the greater risk of severe side effects with increasing total doses, a shorter duration of treatment is normally employed and this in turn leads to a high rate of relapse and treatment failure in chloramphenicol therapy (Svenungsson, 1982).

Trimethoprim-sulfamethoxazole (Cotrimoxazole or septrin) has been found to compare favourably with chloramphenicol in efficacy in the treatment of typhoid fever. It has the advantage of being bactericidal rather than bacteriostatic and so it is expected that there will be fewer carriers and treatment failures with cotrimoxazole (Akinkugbe et al., 1968; Geddes et al., 1971).

With the emergence of chloramphenicol and cotrimoxazole-resistant strains of S. typhi, other antibiotics have been used, including ampicillin, amoxycillin, ciprofloxacin, ceftriaxone, tetracycline, streptomycin or a combination of two drugs (Mathai et al., 1995). Ciprofloxacin has been used to treat typhoid fever caused by multi-resistant S. typhi which was resistant to other antibiotics. Because of this and by virtue of its cost and an oral route of administration,

ciprofloxacin is now considered the ideal choice of drug in a developing country (Eykyn & Williams, 1987; Gulati et al., 1992).

In the drug management of carriers, Christie (1964) found a prolonged course of ampicillin, given in a high dose to be promising. He administered a dose of 1 gram by mouth and 500mg intramuscularly four times daily for one week and thereafter, 1g three times daily for eleven weeks; with probenecid used alongside on a dose of 1 gram twice daily.

2.7 Antibiotic Sensitivity pattern of Salmonella

The worldwide use of antibacterial agents, including antibiotics has been paralleled by the increase in numbers of resistant strains in clinical isolates, offering new problems and challenges. Drug resistance in organisms was previously thought to be solely as a result of chromosomal mutation and selection. With the discovery of the transferable nature of antibiotic resistance in Japan in 1959, it was shown that drug resistance can also be transferred genetically through the transfer of episomes or plasmids. Such plasmids are known as Resistance - factors or R-factors (Mitsuhashi, 1971). Cell to cell contact was soon shown to be necessary for transfer of drug resistance. Once a drug resistant organism has emerged in a bacterial population, the resistance can be transferred to other cells by the mechanisms of transformation, transduction, or conjugation, depending on the type of mechanism which is applicable to the particular bacteria

(Jawetz et al., 1984).

Some R-factors contain in addition to the resistance gene(s), a resistance transfer factor (RTF) which codes for the transfer of the episome to other cells by conjugation. These R-factors are said to be transmissible. The RTF may be an independent episome that gets recombined with an R-factor (Mackie & McCartney, 1978).

An R-factor may contain genes that confer resistance to several antibiotics simultaneously. A typical example is the R-factor that transfers multiple drug resistance among Enterobacteriaceae. This has occurred from Escherichia coli to Shigella flexneri and from Escherichia coli to Salmonella species (Pike, 1975). Some R-factor containing Enterobacteriaceae responsible for outbreaks of disease are chloramphenicol - resistant Salmonella typhi (Olarte and Galindo, 1973), a variety of Salmonella, and all species of Shigella (Ewing, 1986).

R-factors carrying chloramphenicol resistance was first identified in S. typhi in 1972. Since then, epidemics of chloramphenicol - resistant typhoid fever in Mexico and Asia have had higher mortality rates and have proved more difficult to control (Mackie & McCartney, 1978).

In a review, Rowe and Threlfall, (1984) concluded that multiple-drug resistance is an inevitable consequence of the use of antibiotics in human and veterinary medicine. It can be controlled only if international efforts are made to

minimize the unnecessary use of these drugs. Resistant strains do not compete well in the absence of selective antibiotics and usually will disappear when the use of the antibiotics to which they are resistant is discontinued.

In view of the problem of drug resistance, it is important to establish the susceptibility of the S. typhi strains to the antibiotic used for treatment. The disk diffusion method is still the moist widely used for the routine sensitivity testing of isolates from patients. Several modifications of the test have been advocated which vary in their methods of standardization and interpretation (Mackie & McCartney, 1978). The Kirby-Bauer Method (Bauer et al., 1966), is the official method of the USA Food and Drugs Administration. Disks of a single, usually high content of antibiotic are placed on an inoculum of strictly standardized density on Mueller Hinton agar. Three grades of sensitivity are recognized -sensitive, intermediate and resistant - by comparing the diameters of the inhibition zones with critical zone diameters in published tables.

2.8 Control of typhoid fever

Since typhoid fever has an oral-faecal transmission and its occurrence is related to unhygienic circumstances, it has not been possible to control it in the developing countries where low standards of hygiene prevail, especially among the rural dwellers (Ivanoff et al., 1994). Christie (1974) observed that the disease should be controlled by the

application of the principles of hygiene and public health such as the provision of pure water, pasteurized milk supply and a water carriage sewage system. He added that these, coupled with a strict supervision of the preparation and distribution of food in public eating places, will reduce the incidence of typhoid fever to a minimum.

In a seminar paper, Okolacha & Kabir, (1994) cited public health education, especially among the rural dwellers, as a very important tool in the control of typhoid and paratyphoid fevers. They maintained that the provision of health educational facilities is of vital importance in many human settlements because much of ill-health is due to ignorance of simple precautions that can be taken within the circumstances of the rural communities. They observed that the success of any scientific control method depends on awareness in the community, of animal and human disease hazards and this can only come through educat0ion in the basic problems.

On the management of cases and carriers, Anderson et al., (1969) stressed the importance of quarantine and personal hygiene in the control of typhoid and paratyphoid fevers. Patients should be sufficiently segregated to prevent the infectious discharges from reaching other persons. Carriers should be refrained from handling food for the consumption of others except those members of their households who have been

recently immunized. Health departments should keep records of all known carriers and visit them periodically to make sure that they are observing these precautions. Carriers should be followed up and cultures of their faeces or urine obtained periodically until they stop excreting the organism or a surgical removal of the focus of infection.

On immunization, it is necessary to prepare vaccines which could be used as a public health tool in the prevention of typhoid fever in developing countries (Ivanoff et al., 1994). Gupta et al., (1990) emphasised that the introduction of vaccination programs is necessary in areas where antibiotic resistance to first line drugs is widespread or where conditions are ripe for an epidemic.

A parenteral vaccine containing a mixture of heat-killed typhoid and paratyphoid A and B organisms has been used since 1896. Because of its reactogenicity, research has been oriented towards vaccination orally using live organisms and purified antigen. A live vaccine, Ty21a, given by oral route in a triple dose and a highly purified Vi polysaccharide vaccine have been extensively tested and found to confer immunity for at least seven and three years respectively (Cryz et al., 1993; Ivanoff et al., 1994). The Ty21a vaccine has been licensed for human use (Kollaritsch et al., 1996). New live vaccines given orally in one dose have been constructed through genetic engineering (Ivanoff et al., 1994).

Justification

Typhoid fever continues to be a global health problem. In Nigeria as in many other developing countries the incidence of human salmonellosis has not been widely determined. Indeed it is difficult to estimate its worldwide impact because the clinical picture resembles many other febrile infections, and because of the limited capacity for bacteriological diagnosis in most areas of the developing countries owing to lack of funds (Ivanoff et al., 1994).

In the past decade, research work on typhoid fever in Nigeria and in Zaria in particular has been lacking. Studies carried out by Akoh (1987) and Bala & Auty (1977) centered on the clinical pattern of typhoid fever in Zaria. To our knowledge there is no recently documented study on the epidemiology of typhoid fever in Zaria. Coupled to this is the acute dearth of recent literature on typhoid fever from the developed countries because of the diminishing importance of typhoid fever in these countries. The present study was therefore necessary to provide data on the current epidemiology of typhoid fever in Zaria.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Hospital - Based Sampling and Screening

Patients attending various hospitals in Zaria were sampled. The hospitals used were University Health Services Center, A.B.U., Samaru Campus, Jama'a Hospital, Samaru, Ahmadu Bello University Teaching Hospital, Tudun Wada, Zaria Clinic, Tudun Wada, Limi Hospital, Sabon Gari, St. Lukes Anglican Hospital, Wusasa and General Hospital, Kofan Gaya, Zaria City. The sampling was carried out from 5th May to 30th October, 1996. The patients sampled were those reporting to the hospitals' diagnostic laboratories with febrile illnesses or abdominal upset. Questionnaires were administered and samples collected from the patients.

3.1.1. Questionnaires

Questionnaires were administered to the patients by oral interview to obtain information on patients' sex, age, place of domicile, occupation, socio-economic status previous vaccination against typhoid, self-medication as well as symptoms of their illness. Occupation and availability of pipe borne water and water closet toilet were used as measures of socio-economic status. Information obtained was entered into a proforma sheet.

3.1.2 Widal Agglutination Tests

Three to five milliliters of venous blood was collected from each patient into a sterile screw-capped test tube and

allowed to clot for 30 minutes and then centrifuged in a Bench Centrifuge (B&T Model (Sfarle Company) at 2000 rpm for 5 minutes, to obtain sera for the test. The sera wer stored in a refrigerator at 4°C until they were used. Before they were used, the test tubes were removed from the refrigerator and kept at room temperature until the sera thawed.

Salmonella typhi 'O' and 'H' antigen suspensions obtained from Micropath Company, Omega Diagnostics, United Kingdom, were used.

The slide agglutination test was employed as an initial screening test to detect the presence of antibody in the patients' sera. Thereafter, microtitration was carried out on the sera that showed agglutination in the preliminary slide test.

The sera were serially diluted with phosphate-buffered saline (PBS, pH 7.8). A series of doubling dilutions of the sera were prepared in the wells of a microtitre plate using a 0.1ml blow out pipette. This was done according to the method of Lynch et al. (1969), whereby 0.09ml of PBS was delivered into the first well in a row and 0.05ml into each of the next seven wells.

About 0.01ml of a test serum was then added to the first well, the contents mixed and 0.05ml of the mixture was transferred to the second well. Dilution was continued in this way up to the seventh well from which 0.05ml of the mixture was discarded. These gave dilutions ranging from 1 in

10 in the first well to 1 in 640 in the seventh well. The eighth well served as a control containing only PBS. The agglutination was carried out by adding about 0.05ml of antigen suspension to all the test wells. The microtitre plate for the 'O' antigen was incubated in a water bath at 56°C for 4 hours and then left at room temperature overnight before observation under a low power objective lens for agglutination. The 'H' plate was incubated at 37°C for 2 hours and then observed for agglutination.

The titre was taken as the reciprocal of the highest dilution in which agglutination occurred. In this study, an anti-'O'-antibody titre of ≥ 160 was used as a criterion for presumptive positive diagnosis (Raphael & Spencer 1983).

3.2 Bacteriological Culture for the Isolation of S. typhi

Stool and urine samples were collected from the patients whose blood samples were taken for Widal agglutination tests. A total of 500 samples comprising 382 stool and 118 urine were collected. The samples were collected in sterile screw-capped universal bottles and transferred to the laboratory within one hour for analysis. Where it was not possible to transport the samples to the laboratory immediately after collection, they were kept in a refrigerator until they could be transferred to the laboratory. In the laboratory the following procedures were used for the isolation of Salmonella.

3.2.1 Selective enrichment

About 1 gram of faeces was enriched in 9ml of Mannitol Selenite broth in a universal bottle. Urine samples were centrifuged at 2000 rpm for 15 minutes in a Bench Centrifuge (B & T Model from Sfarle Company). The top liquid was decanted using a Pasteur pipette and about 1 ml of the remaining liquid was enriched as for the faecal samples. These were incubated at 37°C for 18-24 hours.

3.2.2. Selective and differential plating

The cultures from the enrichment broth were plated on MacConkey agar and Bismuth Sulphite agar (BSA). All the plates were incubated at 37°C for 18-24 hours (Koneman et al., 1979).

3.3 Biochemical Characterization

After incubation, one or more well separated colonies appearing as Salmonella were picked from the plating media and subjected to preliminary biochemical tests by inoculating onto urea agar slants and Triple Sugar Iron (TSI) media. Colonies that were Urease-negative and gave an alkaline/acid reaction on TSI with or without production of Hydrogen Sulphide (H_2S) were stored on nutrient agar slants at 4°C until complete biochemical characterization was carried out.

Isolates giving the typical biochemical reactions of Salmonella were identified and subjected to serological typing.

3.4 Serological Tests

Serotyping was carried out in order to confirm the isolates biochemically identified as Salmonella. A commercially prepared Salmonella 'O' Polyvalent antiserum (Sigma Co. Ltd) was used in the slide agglutination technique.

Before being tested, isolates were transferred onto Nutrient agar plates and incubated overnight at 37°C. Using a sterile wire loop, a pure colony of isolate was picked from the Nutrient agar plate and emulsified in a drop of sterile normal saline placed on a clean glass slide. A drop of the polyvalent anti-serum was then added to the emulsion and then mixed. The glass slide was rocked for 2-3 minutes and then observed for agglutination. Isolates showing agglutination were confirmed as Salmonella organisms.

Due to lack of specific monovalent antisera, species identification was based on biochemical reactions.

3.5 Biochemical Differentiation of Salmonella serotypes

Isolates confirmed to be Salmonella by serological tests with Salmonella 'O' polyvalent antiserum were subjected to further biochemical tests in other to disccriminate between the different serotypes (Ewing, 1986).

3.6 Antimicrobial Susceptibility Testing

All the S. typhi isolates were tested for their susceptibilities to various antimicrobial agents using the Method of Bauer et al. (1966). Sensidiscs obtained from

Becton Dickinson Microbiology Systems, Cockeysville, MD USA, containing the following concentrations of antibiotics were used:- Chloramphenicol (30μg), Flucloxacillin (5μg), Gentamicin (10μg), Cefuroxime (30μg), Ceftazidime (30μg), Amoxicillin/Clavulanic acid (30μg), Azithromycin (15μg), Ampicillin/Sulbactam (20μg) and Trimethoprim-Sulfamethoxazole (25μg).

One colony of each isolate was inoculated into 5ml of Trypticase Soy broth. The broth cultures were incubated at 35°C for 2-6 hours to develop a turbidity equivalent to the 0.5 McFarland turbidity Standard which was prepared by adding 0.5ml of a 1.175% $BaCl_2$ $2H_2O$ solution to 9.5ml of 1% sulphuric acid (Chesbrough, 1985).

A sterile cotton swab was dipped into the inoculum and the excess fluid expressed by rotating it several times on the inside wall of the tube. This was used to streak the entire agar surface of a Mueller Hinton Agar plate, turning the plate 60° between streakings to obtain even inoculation. The plates were left open on the table for 5 minutes to allow surface moisture to be absorbed and then the discs were applied using sterile forceps and pressed down to make contact with the surface. Within 15 minutes the plates were incubated, in an inverted position, at 35°C.

After 16-18 hours, the plates were examined and diameters of zones of inhibition were measured to the nearest millimeter. The results were interpreted based on the zone

diameter interpretive standards provided by the manufacturers of the Sensi-discs (See Appendix II).

3.7 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of some of the antibiotics to which the isolates showed susceptibility were determined according to the method of Baron and Finegold, (1990). Three antibiotics were used, namely Chloramphenicol, Ampicillin-Sulbactam and Trimethoprim-Sulfamethoxazole.

A set of six test tubes was prepared, each containing 1ml of Nutrient broth. Serial dilution of the test antibiotic was first carried out as follows: 1ml of the concentrated antibiotic solution (500μg/ml) was added to the first tube and mixed. 1ml of the resulting solution was transferred to the second tube and mixed. Serial dilution was continued in this way until the last tube from which 1 ml of the solution was discarded. The resulting dilutions gave concentrations of 250μg/ml, 125μg/ml, 62.5μg/ml, 31.25μg/ml, 15.62μg/ml and 7.8μg/ml respectively.

One ml of standard incoculum of the test organism ($3x10^{16}$ cell/ml) was then inoculated into each tube and to a tube of the growth medium without antimicrobial agent which served as a control. The bacterial suspension was thus diluted to $1.5x10^{6}$ cells/ml and the antibiotic solutions to the concentrations of 125μg/ml, 62.5μg/ml, 31.25μg/ml, 15.6μg/ml, 7.8μg/ml and 3.9μg/ml respectively.

The tubes were incubated at 37°C for 24 hours before being read. An unicoculated tube of growth medium was also incubated to serve as a negative control. After incubation, the tubes were examined for turbidity which indicated growth of the organism.

The MIC was recorded as the lowest concentration of antibiotic that inhibited the growth of the isolates, as detected by lack of visual turbidity.

CHAPTER FOUR

RESULTS

4.1 Screening/Sampling of Patients

A total of 500 patients were examined for S. typhi and S. typhi 'O' and 'H' agglutinins over a period of six months from 5th May to 30th October, 1996. They consisted of 249 males and 251 females whose ages ranged between 1 and 65 years.

4.1.1 Widal agglutination tests

A total of 500 blood samples were collected for the Widal agglutination tests. Eighty-two (16.4%) of the samples gave positive reactions with titres of ≥ 160 against the S. typhi 'O' antigen and 136 (27.2%) samples gave titres of ≥ 160 against the S. typhi 'H' antigen. Twenty-two (4.4%) samples gave titres of ≥ 160 against both 'O' and 'H' antigens, while 418 (83.6%) samples gave titres of 'O' agglutinins less than 160 and 364 (72.8%) samples gave titres of 'H' agglutinins less than 160. (See Appendix 1).

4.1.2 Biochemical Characterization of Isolates

A total of 500 samples comprising 382 stool and 118 urine were collected and cultured for isolation of Salmonella. Ninety-nine isolates which appeared as Salmonella on the growth media, and gave positive reaction in the preliminary tests were subjected to further biochemical characterisation.

Based on the biochemical reactions, 57 isolates were suspected to be members of the Samonella group of organisms.

Other genera suspected on the basis of biochemical reactions were Arizona (10 isolates), Shigella (23 isolates) and Proteus (9 isolates).

4.2 Results of Serological typing

The serological typing using Salmonella 'O' polyvalent and antiserum confirmed 57 of the isolates as belonging to Salmonella (Table 2).

4.3 Biochemical Differentiation of Salmonella Serotypes

Fifty-seven isolates, confirmed to be members of the genus Salmonella were differentiated based on further biochemical characterisation. The results are shown on Table 2.

Table 3 shows the number and percentage of S. typhi and S. paratyphi isolates from the stool and urine samples. S. typhi and S. paratyphi were isolated from 36 or 7.2 of the samples. S. typhi alone was isolated from 23 or 4.6% of the samples and paratyphi A and B from 6 and 7 of the samples respectively, giving a total of 13 isolats or 2.6%.

Twenty-one of the S. typhi isolates were from stool and two from urine samples, four of S. paratyphi A from stool and two from urine samples, seven of S. paratyphi B from stool and none from urine samples (Table 3).

The cases of typhoid and paratyphoid fevers were taken to be those patients whose positive Widal reactions were confirmed by isolation of S. typhi or S. paratyphi.

4.4 Specific Rates

4.4.1 Monthly Frequency

The monthly frequency of isolation of *S. typhi* and *S. paratyphi* for the six months covered by the study is shown in Table 4. The highest prevalence occurred in May and August with rates of 11.1% and 12.7% respectively. Thereafter the prevalence decreased to 4.4% in September and 1.8% in October. In general, the prevalence rose to a peak in August and thereafter declined. This is illustrated in Figure 1.

4.4.2. Age and Sex-specific Rates

Table 5 shows the distribution of the cases by age and sex. The highest isolation frequency occurred in the 11-20 years age group with a rate of 15.0%, followed by the 21-30 years age group with a rate of 11.8%. The age group of 31-40 showed the lowest rate of 1.7%, followed by the 0-10 years age group with the rate of 2.4%. These results indicate that individuals aged between 11 to 30 years are in highest risk of typhoid and paratyphoid fevers, while the young children under 10 and adults above 30 years of age are less frequently infected.

The 36 cases of typhoid and paratyphoid fevers comprised 21 males and 15 females. This gives a male to female ratio of 1.4:1.0, indicating that males are slightly more frequently infected than females (Figure 2).

4.4.3 Socio-economic Status

Tables 6 and 7 show the distribution of the cases according to socio-economic status. Table 6, shows the number of cases from different occupational groups. Ten (5.7%) were civil servants, four (4.16%) were businessmen, twelve (9.67%) were traders and ten (9.43%) were farmers and pastoralists. The highest prevalence occurred in the group of traders, followed by farmers and pastoralists.

Availability of amenities such as pipe-borne water and water closet toilet or use of well water and pit latrine served as further measures of socio-economic status. Table 7 illustrates the distribution of these amenities among the cases. Out of the 36 cases, only thireen (5.0%) had access to pipe-borne water while twenty-three (10.5%) obtained their drinking water from wells. Fourteen cases (4.6%) used water closet toilets and twenty-two cases (11.1%) used pit latrines. For the traders and farmers, the average frequency of isolation was 9.6% while the average frequency for the civil servants and businessmen was 4.93%. The average frequency for the group that obtained their drinking water from wells and used pit latrines of isolation was 10.8% and for those that used pipe-borne water and water closet toilet 4.8%.

Figure 3 and 4 show the distribution of the cases according to the various socio-economic levels. Traders and farmers/pastoralists had the highest frequency of typhoid and paratyphoid fevers as do those that obtained their drinking

water from wells and used pit latrines.

4.5 Disease experience

The symptoms of the disease which were observed in the cases are shown in Table 8. Thirty four (94%) of the cases experienced fever, 25(69%) had headache, 13(36%) had general body pains and 31(86%) had abdominal pain. Diarrhoea and vomiting, occurred in 9(25%) and 10(28%) of the cases respectively, mainly in the cases from whom S. paratyphi B were isolated. One patient (3%) was delirious and one (3%) was in coma. The most frequent combination of symptoms was fever, headache and abdominal pains.

Out of the 500 patients sampled, 280, including 25 of the typhoid cases, had started treatment with antimalarial drugs before attending hospital and 33 had started treating themselves with Chloramphenicol or Amoxicillin.

Only two out of the 500 patients sampled had been previously vaccinated against typhoid fever.

4.6 Susceptibility Pattern of S. typhi Isolates

The 23 S. typhi isolats were tested for their susceptibility to 9 antimicrobial agents and the results are shown in Table 9.

The isolates showed three patterns of sensitivity as follows: Fifteen isolates were sentitive to 8 drugs, five isolates were sensitive to 7 drugs and three isolates were sensitive to 6 drugs. Isolates showing intermediate sensitivity were taken to be resistant to the antibiotic.

Overall, all the 23 S. typhi isolates were sensitive to five of the antibiotics - Chloramphenicol, Gentamicin, Ceftazidime, Ampicillin-Sulbactam and Co-trimoxazole. 15 isolates were sensitive to Flucloxacillin and Cefuroxime, 7 isolates were sensitive to Azithromycin and 21 isolates were sensitive to Amoxicillin/Clavulanic acid.

Multiple resistance was exhibited by 8 of the isolates, five of which showed resistance to both Flucloxacillin and Cefuroxime, one to Flucloxacillin, Cefuroxime and Azithromycin and two to Flucloxacillin, Cefuroxime and Amoxicillin-Clavulanic acid (Table 9).

Tables 10 and 11 show the minimum inhibitory concentrations (MICs) of 3 of the antibiotics for the 21 isolates. For Chloramphenicol, MIC was 15.6μg/ml for one isolate, 7.8μg/ml for five and 3.9μg/ml for seventeen isolates. For Ampicillin-Sulbactam the MIC was 5.6μg/ml for ten isolates, 7.8μg/ml for 11 isolates and 3.9μg/ml for two isolates. For Cotrimoxazole, the MIC was 15.6μg/ml for seventeen isolates, and 7.8μg/ml for six isolates.

TABLE 1

Data on Patients Testing Positive for S. typhi 'O' agglutionins (≥160) and Culture

	S.typhi 'O'	S. typhi	S. paratyphi A	S. paratyphi B
Sex:				
M	47	14	3	4
F	35	9	3	3
AGE Group (Years)				
0 - 10	12	1	0	1
11-20	31	12	2	2
21-30	26	8	1	3
31-40	5	1	1	0
above 40	8	1	2	1
Place of Domicile:				
SM	18	5	0	3
TW	8	4	1	2
Sb	22	4	3	0
Zc	20	9	1	1
Bs	2	0	0	0
Ws	12	1	1	1

Table 1 continued

	S.typhi 'O'	S. typhi	S. paratyphi A	S. paratyphi B
Occupation CS	35	6	1	3
B	3	0	2	2
Tr	20	9	1	2
FM & Ps	24	8	2	0
SOURCE OF DRINKING WATER				
Pb	30	6	4	3
W	52	17	2	4
Type of Toilet				
Wc	24	6	4	4
P	58	17	2	3

Table 1 continued

	S.typhi 'O'	S. typhi	S. paratyphi A	S. paratyphi B
Symptoms				
Fever	73	23	4	6
Headache	51	18	3	4
GBP	26	6	1	3
Abdominal pain	46	21	6	4
Insomnia	25	9	2	2
Anorexia	27	13	3	0
Weight loss	31	14	3	4
Weakness	29	12	2	1
Diarrhoea	16	1	2	7
Vomiting	12	4	3	5
Constipation	9	6	1	0
Rash	0	0	0	1
Mental Confusion	0	0	0	1
Cough	13	5	1	1
Coma	1	1	0	0

Table 1 continued

	S.typhi 'O'	S. typhi	S. paratyphi A	S. paratyphi B
PREVIOUS MEDICATION.				
Cq	45	16	4	4
Fan	3	1	0	1
Mq	2	1	0	0
TC	2	1	0	0
Clph	2	0	0	0

KEYS * Number of patients positive for each parameter or test

M = Male
F = Female
SM = Samaru
TW = Tudun Wada
Sb = Sabon Gari
ZC = Zaria City
Bs = Basawa
Ws = Wusasa
Cs = Civil Servant
B = Business
Tr = Trader
Fm & Ps = Farmers and pastoralists.

Pb = Pipe borne water
WC = Water closet toilet
W = Well water
P = Pit latrine
Cq = Chloroquine
Fan = Fancidar
Mq = Melaquine
TC = Tetracycline
Clph = Chloramphenicol

TABLE 2

BIOCHEMICAL REACTIONS TO DIFFERENTIATE SALMONELLA SEROTYPES

Isolate Number	SIMMON'S Citrate	Arabinose	Trehalose	Xylose	Glucose A	Glucose G	Inositol	Rhamnose	Lysine decarboxylase	Ornithine decarboxylase	Beta galactosidase	Mucate	Jordan's D-tartrate	SEROTYPE
3	-	-	+	+	+	-	-	-	+	-	-	-	+	S. Typhi
4	-	-	+	+	+	-	-	-	+	-	-	-	+	S. Typhi
22	-	-	+	+	+	-	-	-	+	-	-	-	+	S. Typhi
31	+	+	+	+	+	+	-	+	+	+	-	+	-	S. paratyphiB
37	+	+	+	+	+	+	+	+	+	+	-	+	+	*
54	+	+	+	+	+	+	-	+	+	+	-	+	-	S. ParatyphiB
62	+	+	+	+	+	+	-	+	+	+	-	+	-	S. ParatyphiB
65	+	+	+	+	+	+	+	+	+	+	-	+	+	*
71	+	+	+	+	+	+	+	-	+	+	-	+	+	*
75	+	+	+	+	+	+	-	+	+	+	-	+	-	S. paratyphiB
88	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
94	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
95	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
105	+	+	+	+	+	+	+	-	+	+	-	+	+	*
121	+	+	+	+	+	+	+	-	+	+	-	+	+	*
139	+	+	+	+	+	+	-	+	+	+	+	+	-	S. paratyphiB
145	+	+	+	+	+	+	-	+	+	+	-	+	-	S. paratyphiB
167	-	+	+	-	+	+	-	+	-	+	-	-	-	S. paratyphiA
171	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
177	+	+	+	+	+	+	+	+	+	+	-	+	+	*
183	+	+	+	+	+	+	+	-	+	+	-	+	+	*
188	+	+	+	+	+	+	+	-	+	+	-	+	+	*
196	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
210	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
212	+	+	+	+	+	+	+	-	+	+	-	+	+	*
216	-	+	+	-	+	+	-	+	-	+	-	-	-	S. paratyphiA
226	+	+	+	+	+	+	+	-	+	+	-	+	+	*
236	-	+	+	-	+	+	-	+	-	+	-	-	-	S. paratyphiA
241	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
243	-	+	+	-	+	+	-	+	-	+	-	-	-	S. paratyphiA
253	+	+	+	+	+	+	+	-	+	+	-	+	+	*
264	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
267	+	+	+	+	+	+	+	+	+	+	-	+	+	*
270	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
276	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
290	+	+	+	+	+	+	+	-	+	+	-	+	+	*
296	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
300	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
307	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
311	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
312	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
316	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
322	+	+	+	+	+	+	+	+	+	+	-	+	+	*
329	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
337	+	+	+	+	+	+	+	+	+	+	-	+	+	S. typhi
343	-	+	+	-	+	+	-	+	-	+	-	-	-	S. paratyphiA
347	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
368	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
371	+	+	+	+	+	+	+	+	+	+	-	+	+	*
381	+	+	+	+	+	+	+	+	+	+	-	+	+	*
419	-	+	+	-	+	+	-	+	-	+	-	-	-	S. paratyphiA
425	-	-	+	+	+	-	-	-	+	-	-	-	+	S. typhi
458	+	+	+	+	+	+	+	-	+	+	-	+	+	*
471	+	+	+	+	+	+	+	-	+	+	-	+	+	*
482	+	+	+	+	+	+	-	+	+	+	-	+	-	S. paratyphiB
488	+	+	+	+	+	+	+	+	+	+	-	+	+	*
493	+	+	+	+	+	+	+	-	+	+	-	+	+	*

A = Acid
G = Gas
* = Other Salmonella serotypes

TABLE 3

TABLE 3

Number of S. typhi and S. paratyphi isolates from stool and Urine Samples

Type of Sample	Number Collected	Number of Isolates S. typhi	S. paratyphi A	S. paratyphi B
Stool	382	21	4	7
Urine	118	2	2	0
Total	500	23(4.6%)	6(1.2%)	7(1.4%)

TABLE 4

Monthly Frequency of Typhoid Fever

Month	Number of patients examined	Number &(%) positive for Widal reaction (0≥160)	Number positive for: S. typhi	S. paratyphi	Total
May	36	8 (22.2)*	3	1	4 (11.1)
June	118	16 (13.6)	3	5	8 (6.8)
July	96	20 (20.8)	4	4	8 (8.3)
August	79	19 (24.1)	10	0	10 (12.7)
Sept.	114	12 (10.5)	3	2	5 (4.4)
October	57	7 (12.2)	0	1	1 (1.8)
Total	500	82 (16.4)[1]	23 (4.6)	13 (2.6)	36 (7.2)

* Figure in parenthesis indicate the monthly specific rates (percent)

1 Prevalence rates for the six months study period.

TABLE 5

Age - Sex Distribution of Cases ofTyphoid and Paratyphoid Fevers

Age Group (Years)	Male		Female		Total for Age	
	Number examined	Number & percentage positive	Number examined	Number percentage	Number examined	Number positive
0-10	33	1 (3.0)*	49	1 (2.0)	82	2 (2.4)
11-20	71	11 (15.7)	36	5 (13.5)	107	16 (15.0)
21-30	54	7 (13.0)	48	5 (10.0)	102	12 (11.8)
31-40	43	0 (0.0)	57	2 (3.5)	100	2 (2.0)
Above 40	48	2 (4.2)	61	2 (3.3)	109	4 (3.6)
Total (Sex)	249	21	251	15	500	36

* Figures in parenthesis indicate percent age specific prevalence

TABLE 6

Distribution of Cases of Typhoid and Paratyphoid Fevers According to Occupation in Zaria During Period of May-October, 1986

Occupation	Civil Servant	Businessmen	Trader	Farmer and Pastoralist
Number Examined	174	96	124	106
Number Positive (cases)	10	4	12	10
Percent Frequency	5.70	4.16	9.67	9.43

TABLE 7

Distribution of Cases of Typhoid and Paratyphoid Fevers

According to Welfare Status

	Pipeborne Water (Pb)	Well Water (W)	Water Closet Toilet (WC)	Pit Toilet (P)
Number Examined	283	217	301	199
Number Positive (Cases	13	23	14	22
Percent Frequency	5.0	10.5	4.6	11.1

(Measures of Welfare Status used here are Availability of Pipeborne Water (Pb) and Water Closet toilet (WC) and use of Well Water (W) and Pit Toilet (P)).

TABLE 8

Occurrence of Symptoms among the 36 Cases of Typhoid and Pratyphqoid Fevers

Symptom	Number (and %) of Cases Presenting each Symptom
1. Fever	34 (94)
2. Headache	25 (69)
3. General body pain	13 (36)
4. Abdominal pain	31 (86)
5. Insomnia	15 (42)
6. Anorexia	15 (42)
7. Weight Loss	20 (42)
8. Weakness	14 (39)
9. Diarrhoea	9 (25)
10. Vomiting	10 (28)
11. Constipation	5 (14)
12. Rash	1 (3)
13. Mental confusion (delirium)	1 (3)
14. Cough	7 (19)
15. Coma	1 (3)

TABLE 9

RESULTS OF ANTIMICROBIAL SUSCEPTIBILITY TESTING OF

S. TYPHI ISOLATES

Isolate NO	C-30	FU-5	GM-10	CXM-30	CAZ-30	AMC-30	AZM-30	SAM-20	SXT-25
3	S	S	S	S	S	S	R	S	S
4	S	S	S	S	S	S	R	S	S
22	S	S	S	S	S	S	R	S	S
88	S	S	S	S	S	S	R	S	S
94	S	R	S	I	S	S	S	S	S
95	S	S	S	S	S	S	R	S	S
171	S	S	S	S	S	S	R	S	S
196	S	R	S	I	S	S	R	S	S
210	S	R	S	I	S	S	S	S	S
241	S	S	S	S	S	S	R	S	S
264	S	S	S	S	S	S	R	S	S
270	S	S	S	S	S	S	R	S	S
276	S	R	S	I	S	I	S	S	S
296	S	R	S	I	S	S	S	S	S
300	S	R	S	S	S	S	S	S	S
307	S	S	S	I	S	S	R	S	S
311	S	R	S	I	S	I	S	S	S
312	S	R	S	S	S	S	S	S	S
316	S	S	S	S	S	S	R	S	S
329	S	S	S	S	S	S	R	S	S
347	S	S	S	S	S	S	R	S	S
368	S	S	S	S	S	S	R	S	S
425	S	S	S	S	S	S	R	S	S

Keys
C - Chloramphenicol
Fu - Flucloxacillin
GM - Gentamicin
CXM - Cefuroxime
CAZ - Ceftaxidime
AMC - Amoxicillin/Clavulanic acid
AZM - Azithromycin
SAM - Ampicillin-Sulbactam
SXT - Trimethoprim-Sulfamethoxazole

S - Sensitive
R - Resistant
I - Intermediate

TABLE 10

RESULTS OF MINIMUM INHIBITORY CONCENTRATION (MIC) TESTS ON *S. TYPHI* ISOLATES.

Number of Isolate	Chloramphenicol	Ampicillin -Sulbactam	Trimethoprim-Sulfamethoxazole
3	7.8 µg/ml	7.8 µg/ml	15.6 µg/ml
4	7.8 "	15.6 "	15.6 "
22	15.6 "	15.6 "	15.6 "
88	3.9 "	15.6 "	7.8 "
94	3.9 "	15.6 "	15.6 "
95	3.9 "	15.6 "	15.6 "
171	3.9 "	7.8 "	15.6 "
196	3.9 "	7.8 "	7.8 "
210	3.9 "	3.9 "	15.6 "
241	3.9 "	7.8 "	15.6 "
264	7.8 "	7.8 "	15.6 "
270	3.9 "	7.8 "	15.6 "
276	3.9 "	3.9 "	7.8 "
296	3.9 "	15.6 "	15.6 "
300	7.8 "	15.6 "	15.6 "
307	3.9 "	7.8 "	15.6 "
311	3.9 "	7.8 "	7.8 "
312	3.9 "	15.6 "	15.6 "
316	3.9 "	7.8 "	7.8 "
329	3.9 "	7.8 "	15.6 "
347	3.9 "	7.8 "	7.8 "
468	7.8 "	15.6 "	15.6 "
525	3.9 "	15.6 "	15.6 "

TABLE 11

Minimum Inhibitory Concentrations (MICs) of Chloramphenicol, Ampicillin-Sulbactam and Trimethoprim-Sulfamethoxazole with the Number of *S. typhi* isolates for each Antibiotic.

Antibiotics	Chloramphenicol	Ampicillin-Sulbactam	Trimthoprim/ Sulfamethoxazole
Minimum Inhibitory Concentration (MIC) µg/ml	15.6 (1*)	15.6 (10)	15.6 (17)
	7.8 (5)	7.8(11)	7.8 (6)
	3,9(17)	3.9(2)	3.9 (0)

* Figures in parenthesis represent number of isolates.

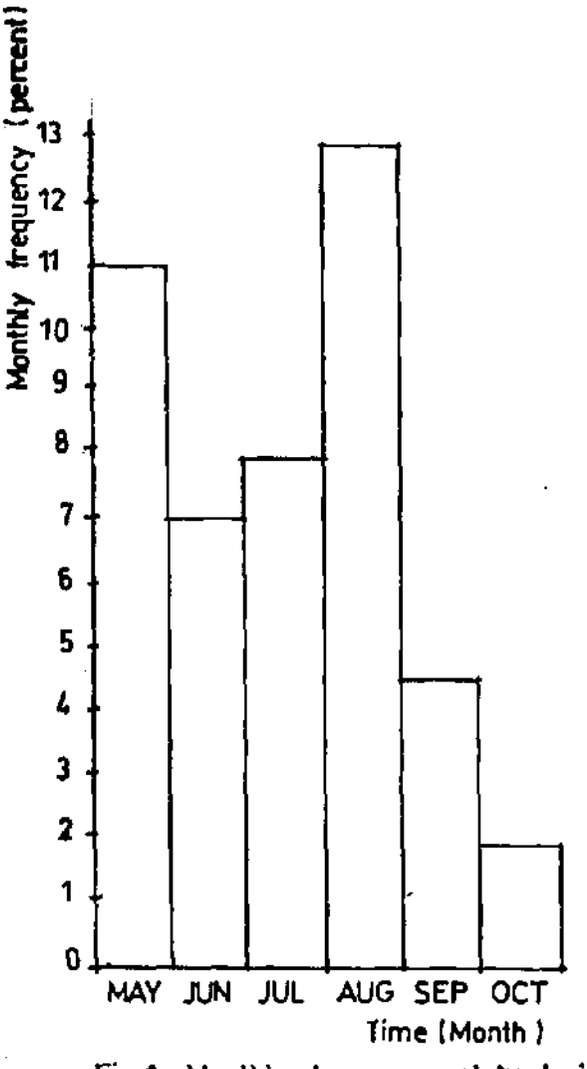


Fig 1 Monthly frequency of typhoid and Paratyphoid fevers

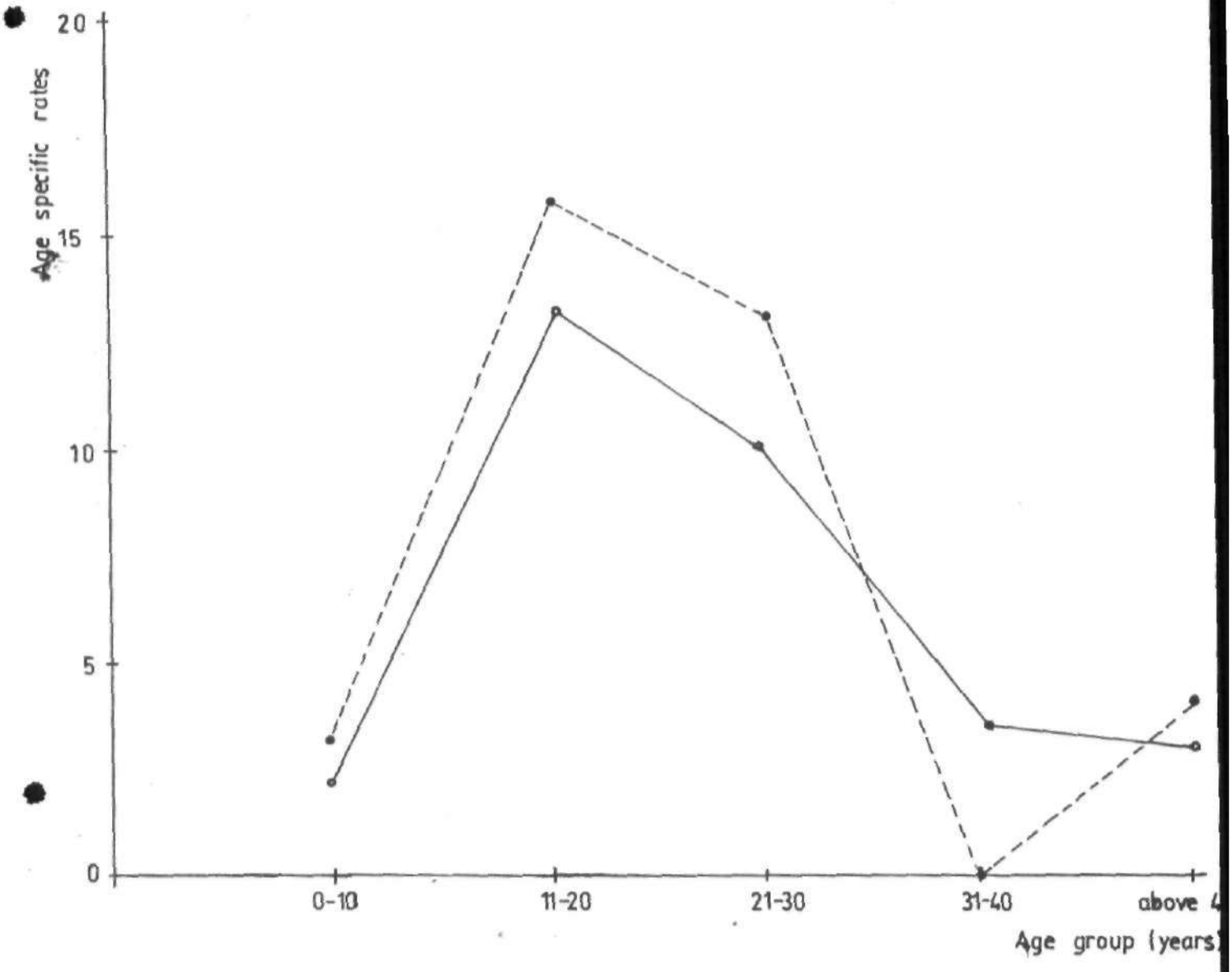


Fig 2 Age and sex distribution of cases of typhoid and Paratyphoid fevers

Key: ---------• Males

---------o Females

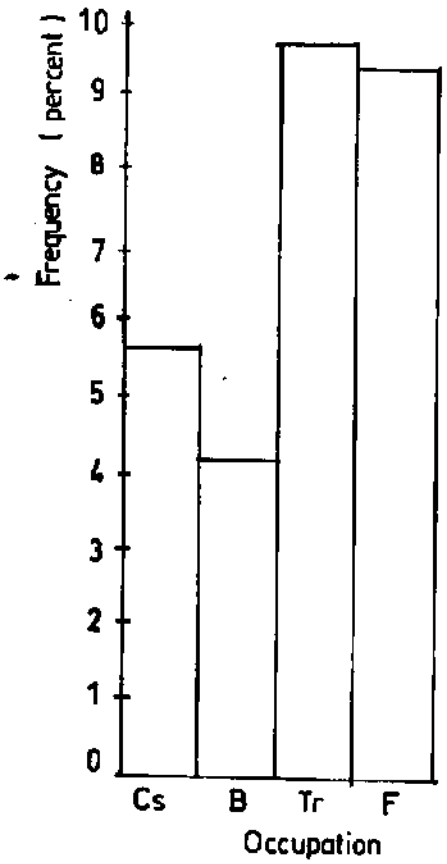


Fig 3 Distribution of cases according to Socio economic status

Cs=Civil servants

B=Businessmen

Tr=Traders

F=Farmers

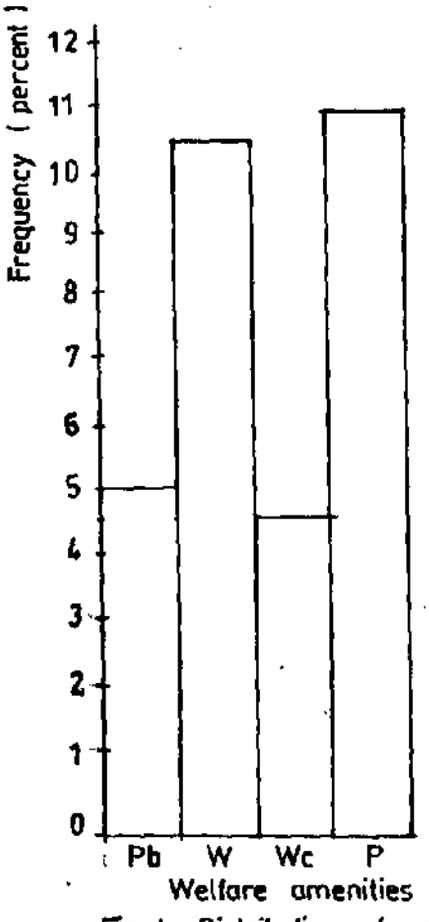


Fig 4 Distribution of cases according to Socio ecomonic status

Pb = Pipeborne water
W = Well water
Wc = Water closet
P = Pit latrine

CHAPTER FIVE

DISCUSSION

In this study, 23 isolates of S. typhi and 13 S. paratyphi were identified. These represent 4.6 percent and 2.6 percent isolation respectively, giving a total prevalence of 7.2 percent. This prevalence is higher than that reported by Okolocha et al (1993) for the same study area in which the average incidence rate over a period of ten years (1983 - 1992) was 2.6 percent. The findings in the present study support the observations of Ivanoff et al., (1994), that the incidence of typhoid fever is high in many developing countries and appears to be increasing over a period of time. The high prevalence reported in the present study is also in agreement with the assertions of Bala et al., (1977) and Akoh (1987) that typhoid fever is common in Zaria.

Four or 11.1% of the isolates were from urine while thirty-two or 88.9% were from stool samples. This is in line with the observations of Mathai et al., (1995) that S. typhi is rarely recovered from urine, even in areas endemic for the disease.

Eighty-two or 16.4(%) of the blood sampled gave positive Widal agglutination reactions with 'O' agglutinin titre of ≥160. The 7.2 percent rate of isolation of S. typhi and S. paratyphi is lower than the rate of positive Widal reactions. The high rate of positive Widal reactions may be due to a

number of factors including a history of previous infection, the possibility of previous subclinical infection, and a history of immunization, all of which may give rise to high levels of S. typhi 'O' and 'H' antibodies in the blood (Raphael & Spencer 1983; Sussman, 1975). 'H' antibodies with titre ≥ 160 were not taken to be significant in this study since the 'H' titre is often non-specific and may rise with any febrile condition (Raphael & Spencer, 1983). Due to the difficulties in interpreting Widal test results (Raphael & Spencer 1983), it has always been necessary to isolate the causal organism from the patients' samples or to demonstrate a two or four-fold rise in antibody titre, before a final diagnosis is made. Because most hospitals in Zaria seem to depend on a single Widal test result for the diagnosis of typhoid fever and no attempts are made to isolate the causative agent, there may be a gross over reporting of typhoid fever cases.

The monthly frequency of typhoid fever within the six months study period varied, with the highest prevalence in May and August. Akoh (1987) conducted a study in Zaria, and reported a clustering of cases during the rainy season (May - October), with two peaks, one coinciding with the onset of rains in May, and the other at the height of rains in August. In agreement with these findings, Archampong (1969), also reported a definite seasonal incidence with two peaks coinciding with the two rainy seasons in Accra, Ghana. In

Lagos, Ogunbiyi and Onabowale (1976), reported three peaks in April, July and November although the disease was present all year round. It seems therefore, that the incidence of typhoid fever may be higher during the rainy season and that contamination of drinking water through flooding could be an important factor in the dissemination of the organism (Ikeme and Anan, 1966). Since most people from the rural areas and slums of Zaria obtain their drinking water from largely unprotected wells which are flooded during the rains, they are at high risk of infection with the causal organism of typhoid fever.

Among the age groups, the highest prevalence occurred in the 11 - 20 year old group with the rate of 15 percent followed by the 21 - 30 year old group with the rate of 11.8 percent. An earlier report by Ogunbiyi and Onabowale (1976) showed that the peak age incidence was in the second and third decades of life. The results of the present study differs slightly from the reports of Bala and Auty (1976), Akoh, (1977) and Okolocha et al., (1993) who noted a peak age incidence in the 0 - 10 year old group in Zaria. The low incidence in the age groups of 0 - 10 years and in persons over thirty years noted in this study has previously been observed by various authors. Ferrecio et al., (1984) noted that the age - specific incidence was low in under three year olds, high among school children aged five to nineteen years and low in adults over thirty-five years. AL- Qurawi et al.,

(1995) observed the absence of patients under one year of age among typhoid fever cases, which could be attributed to the protected life style and role of breast feeding in that age. They also noted an apparent rarity of typhoid fever in children under five years of age which was attributed to the occurrence of mild clinical forms of the disease. The observation on the occurrence of mild forms of typhoid fever in young children agrees with the report of Ferrecio et al., (1984) in which 3.5 percent of children under twenty-four months of age had an unrecognized bacteremia due to S. typhi or S. paratyphi. The low incidence among adults over thirty years has been attributed to acquired immunity (AL- Qurawi et al., 1995).

From the foregoing it seems that although no age group is exempt from the disease, typhoid fever appears to be more prevalent in persons of school age than in persons over thirty years in Zaria.

The 36 cases of typhoid and paratyphoid fevers comprised of 21 males and 15 females, giving a male to female ratio of 1:4:1.0. The results of this study suggests that males are slightly more at risk of infection than females. This observation has been made by various authors (Akoh, 1987; Al Qurawi et al., 1995; Anderson et al., 1969 and Okolocha et al., 1993). No satisfactory reason has as yet been advanced for this, but it may be due to the eating habits of men who tend to eat more freqently outside their homes and also may

not give much attention to the hygienic quality of the food in the various eating places they use.

In this study, two measures of socio-economic status (SES) were used namely occupation and access to welfare amenities. In the case of occupation; the traders, farmers and pastoralists had higher prevalence of the disease than the people in the other occupations which is in line with earlier reports by Okolocha et al., (1993). Individuals who obtained their drinking water from wells and used pit latrines had higher prevalence of typhoid fever than those that had access to good drinking water and water closet toilets. Since these groups who had the higher prevalence of the disease also belong to the low socio-economic class, it may be inferred that people of low socio-economic status are more at risk of typhoid fever than those of high socio-economic status with a low to high socio-economic status ratio of 2.3:1. This is similar to the report of Akoh, (1987) who noted a low to high socio-economic status ratio of 67.19 percent and 10.95 percent in Zaria. The reason for this may be that people of higher socio-economic status are likely to enjoy better sanitation and thus be protected from Salmonella infection. On the other hand, people in the low socio-economic level do not have access to good drinking water and toilet, especially those that live in the slum areas in Zaria where unprotected water wells are wide spread and pit latrines are usually located only a few meters from the dwellings. There is also the

possibility that flies may transfer pathogens on their feet and mouth parts from the pit latrines to food and cutlery. To highlight the importance of environmental sanitation in the transmission of the disease, Ogunbiyi & Onabowale, (1976) showed that 70 percent of typhoid fever patients came from areas characterised by over-crowding and poor sanitation.

Most of the cases of typhoid fever in the present study presented with symptoms of fever, headache and general body pains, signs which are also commonly associated with malaria. Hence, typhoid fever in the early stages was mistaken for malaria and as such more than half of the patients sampled, including 25 of the cases had started treating themselves with various antimalarial drugs before attending hospital. Moreover thirty-three patients treated themselves with chloramphenicol or amoxicillin prior to hospital attendance. This points to a high prevalence of self-medication among patients with febrile illnesses and abdominal upset in Zaria.

Vaccination against typhoid is not common in Zaria. Only two out of the 500 patients sampled have reported being vaccinated against typhoid fever. There is a high rate of ignorance on the issue of vaccination as 99% of the patients in Zaria interviewed did not know that a typhoid vaccine is available.

All the S. typhi isolated in this study showed sensitivity to Chloramphenicol, Gentamicin, Ceftaxidime, Ampicillin-Sulbactam and Cotrimoxazole. Few isolates were

resistant to flucloxacillin, Azithromycin and Cefurozime. Therefore, from the results of the present study, resistance to the common drugs of choice in the therapy of typhoid fever did not occur in S. typhi isolated from clinical samples.

CONCLUSIONS AND RECOMMENDATIONS

- From the findings of the present study, typhoid fever appears to be common in Zaria.
- The age groups of eleven to thirty years were at the greatest risk of infection of S. typhi and S. paratyphi.
- Males were found to be at a slightly higher risk of infection than females.
- Individuals of lower socio-economic level were found to be at higher risk of infection than those of high socio-economic level.
- Contaminated water and inadequate toilet facilities contribute greatly to the transmission and endemicity of typhoid fever in Zaria.
- S. typhi isolates from Zaria were found to be sensitive to the drugs most commonly used in the treatment of typhoid fever namely Chloramphenical, Amoxicillin/Clavulanic acid (Augmentin) and Cotrimoxazole (Septrin).
- Vaccination against typhoid has not been incorporated into the community health programme in Zaria.

In view of the above conclusions it is therefore recommended that:

- there should be school-based vaccination against typhoid since most of those affected are of school age and typhoid vaccination should be included in the community health programme or Expanded Programme on Immunization.

- water wells should be protected and covered adequately from flood and debris in order to prevent contamination of drinking water.
- pit latrines should be adequately covered and located at a safe distance from the living houses to avoid contamination of water, food and cutlery by flies breeding in the latrines.
- work on detection of typhoid carriers should be carried out and good management of carriers incorporated into the health programme.

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APPENDIX 1

Data on The 500 Patients Examined, With Widal Test Results

Sample No.	Month collected	Sex	Age (Yrs)	Occu-pation	Source of drinking Water	Type of toilet	Symptoms of illness	Previous Medication	Previous Vaccination	Widal Test S typhi 'O'	'H'
1	May	M	41	C/s	W	P	1, 3, 8	_	_	*20	80
2	"	F	52	C/s	W	P	1, 3, 11	-	-	20	80
3	"	F	13	St(C/s)	Pb	WC	1,2,4,7,10,15	Cq	-	160	80
4	"	M	14	St(C/s)	W	P	1,4,5,6,7,8,11	Cq	-	160	160
5	"	F	40	C/S	Pb	WC	1,2,3,4	Mq	-	20	20
6	"	F	43	C/S	Pb	WC	1,3,7,11	Cq	-	20	20
7	"	F	31	C/S	Pb	WC	1,2,14	Cq	-	160	160
8	"	M	37	Trader	Pb	P	2,3,11	-	-	160	80
9	"	M	34	C/S	W	P	2,4	Fan	-	20	80
10	"	F	30	Business	Pb	WC	2,3	-	-	20	20
11	"	M	24	St(Farmer)	W	P	1,4,5,8,9	Cq	-	160	160
12	"	M	31	Business	W	P	2,3,5,8,9,14	Cq	-	20	20
13	"	F	48	Farmer	W	P	1,3,7,8	-	-	160	80
14	"	M	32	Trader	Pb	WC	1,2,8,9	-	-	80	160
15	"	M	9	Child(c/s)	Pb	WC	1,4,7,8	Cq	-	20	20
16	"	M	50	C/S	W	P	1,5,6,7,8	-	-	20	80
17	"	F	40	C/S	Pb	Wc	1,8,9	Clph	-	40	160
18	"	M	6½	Child(Trader)	W	C	1,6	Cq	-	20	20
19	"	F	54	C/S	Pb	WC	1,2,8,14	Cq	-	20	20
20	"	M	53	C/S	Pb	WC	1,2,7	Cq	-	20	20
21	"	M	37	C/S	Pb	WC	2,8	Cq	-	20	20
22	"	F	28	Farmer	W	P	1,2,4,8,9,10,14	TC	-	160	80
23	"	F	9	C/S	Pb	WC	1,2,4	Cq	-	160	80
24	"	F	35	C/S	W	P	1,4	Cq	-	20	80
25	"	F	47	C/S	Pb	P	1,2	-	-	20	20
26	"	M	46	C/S	Pb	WC	1,3	Cq	-	20	20
27	"	F	40	C/S	W	P	2,4,7,10	-	-	20	160
28	"	M	42	C/S	Pb	WC	2,4,7	-	-	20	160
29	"	M	35	C/S	Pb	WC	4,10	Cq	-	20	20
30	"	F	34	C/S	W	P	1,4,7	Clph	-	40	40
31	"	F	10	Child(Trader)	W	WC	2,4,9,14	Fan	-	80	20
32	"	M	23	St(Business)	Pb	WC	1,2,11,14	Mq	-	20	20
33	"	F	32	C/S	W	P	1,2,3	-	-	20	160
34	"	M	28	C/S	W	P	1,2,3,6	Cq	-	20	20
35	"	F	38	Trader	W	P	2,6,9	Cq	-	20	160
36	"	M	27	C/s	Pb	WC	2,3,5	-	-	20	160
37	June	F	22	Farmer	Pb	WC	2,4,6,7	Cq	-	160	80
38	"	F	13	St(Business)	Pb	WC	2,3,4	Cq	-	20	20

39	"	M	50	C/S	W	P	1,2,5,6	Fan	-	20	40
40	"	M	29	C/S	Pb	WC	4,6,8,10	-	-	20	160
41	"	F	40	C/S	W	P	1,7,14	-	-	20	20
42	"	F	54	C/S	W	P	7,8,9,10	Cq	-	20	20
43	"	M	19	St(C/S)	Pb	WC	6,8,14	-	-	20	20
44	"	F	19	St(C/S)	W	P	1,2	Cq	-	160	80
45	"	F	32	Business	Pb	WC	3,8	Cq	-	20	20
46	"	M	45	C/S	Pb	WC	1,3,5	-	-	20	20
47	"	M	34	Trader	W	P	1,2,9,10	-	-	40	20
48	"	F	25	C/S	Pb	P	1,4,6,10	Cq	-	160	80
49	"	M	40	C/s	W	P	4,6,9	Clph	-	20	20
50	"	F	21	Tra	Pb	WC	1,2,3,11	Fan	-	80	160
51	"	M	24	St(CS)	Pb	WC	2,4,8	Cq	-	20	20
52	"	M	4	Child(Business)	Pb	WC	1,4,6,7	Cq	-	20	20
53	"	F	10	Child(Trader)	Pb	WC	1,2,4,5	Fan	-	20	20
54	"	M	27½	C/S	Pb	P	1,3,4,5,7,9,10	-	-	20	20
55	"	F	34	Business	Pb	WC	2,3,4,8	Cq	-	20	20
56	"	F	36	C/S	Pb	WC	1,2,4	Clph	-	20	20
57	"	F	2	Child(Business)	W	P	1,8	-	-	80	160
58	"	M	10	C/S	Pb	WC	1,4,5	Cq	-	40	40
59	"	F	8	Child(Trader)	W	P	1,4,8,14	-	-	40	160
60	"	M	44	c/s	W	P	2,4,6,	Fan	-	20	20
61	"	F	35	C/S	Pb	WC	1,14	Cq	-	20	20
62	"	M	17	St(c/s)	W	P	1,4,7,9,13	Cq	-	80	40
63	"	M	14	St(Business)	Pb	WC	1,2,4,7	-	-	40	20
64	"	M	45	Farmer	W	P	2,5,6	Amox	-	20	20
65	"	F	21	St(Business)	W	P	1,9,10,	TC	-	160	80
66	"	M	9	Child(Business)	Pb	WC	1,4	Cq	-	20	20
67	"	F	30	C/s	W	P	1,3,4	-	-	40	40
68	"	M	24	Trader	W	P	6,8	Cq	-	20	20
69	"	M	21	Trader	Pb	WC	1,4,9	Mq	-	80	160
70	"	F	3	Child(Farmer)	W	P	4,8,10	Hal	-	20	20
71	"	M	5	Child(C/S)	W	P	1,5,8,9,10	-	-	160	80
72	"	M	20	Trader	Pb	WC	2,3,8	-	-	20	20
73	"	F	38	C/S	Pb	WC	1,3	-	-	40	160
74	"	M	18	St(Farmer)	W	P	1,2,8,9	Hal	-	20	20
75	"	M	22	St(Business)	Pb	WC	1,4,7,9,10	-	-	40	20
76	"	F	10	Child(Trader)	Pb	WC	1,4,8,9,10	Cq	-	40	20
77	"	F	36	Business	Pb	WC	3,7,8	Clph	-	20	20
78	"	M	13	ST(Farmer)	W	P	1,2,3,8	-	-	160	80
79	"	F	12	St (c/s)	W	P	1,3,9,14	-	-	160	80
80	"	M	40	Business	W	P	1,2	Cq	-	20	80
81	"	F	32	C/S	W	P	2,4,6,8,11	-	-	20	20
82	"	M	39	Pastoralist	W	P	1,2	-	-	40	160
83	"	F	34	Trader	Pb	WC	2,3,4	Amox	-	20	20
84	"	F	32	Trader	Pb	WC	1,2	Cq	-	80	40

85	"	F	19	St(CS)	W	P	1,2,6,11	Cq	-	160	80
86	"	F	40	Business	Pb	WC	1,2.	-	-	20	40
87	"	M	22	C/S	Pb	WC	1,4	Clph	-	20	20
88	"	M	21	Trader	Pb	WC	1,3,4,5,6,7	Cq	-	320	160
89	"	F	32	C/S	W	P	1,4,10	Cq	-	20	20
90	"	F	39	Business	W	P	1,2,4,10	-	-	40	160
91	"	M	36	C/S	Pb	WC	1,4	Cq	-	20	20
92	"	F	25	Trader	Pb	WC	1,4,14	Cq	-	80	40
93	"	M	16	Pastoralist	W	P	1,2,3,4	-	-	40	20
94	"	F	43	C/S	W	P	1,2,3,4,6,7	Cq	-	160	80
95	"	M	8	Child(Farmer)	Pb	WC	1,2,4,5,6,7,8,11	Cq	-	160	160
96	"	F	29	C/S	Pb	WC	2,3	-	-	20	20
97	"	F	24	C/S	Pb	WC	2,4,8	-	-	20	20
98	"	M	49	Business	Pb	WC	1,3	Cq	-	80	160
99	"	M	18	Trader	Pb	WC	2,3,11	Cq	-	20	20
100	"	F	10	C(CS)	Pb	WC	1,2,4,5,8	Cq	-	40	20
101	"	M	16	Pastoralist	W	P	2,5,8	Cq	-	20	20
102	"	F	21	St(CS)	Pb	WC	1,2,3	Cq	-	20	20
103	"	F	2½	Child(Business)	Pb	WC	1,6,8	Cq	-	20	20
104	"	F	16	St(CS)	Pb	WC	1,4,8,9,14	Cq	-	160	80
105	"	M	26	C/S	Pb	P	1,2,5,6,8,9	Mq	-	160	80
106	"	F	35	Trader	Pb	WC	1	Cq	-	20	20
107	"	M	10	Child (C/S)	Pb	WC	2,4,11	-	-	80	160
108	"	F	21	Business	W	P	1,2,3,6,8	Cq	-	20	20
109	"	M	11	Child(Trader)	W	P	1,2,4,14	Cq	-	40	160
110	"	F	29	C/S	Pb	WC	1,2,4,5,6,8	Cq	-	20	20
111	"	F	21	Trader	W	P	1,11	Cq	-	20	20
112	"	F	31	C/S	Pb	WC	4,9	-	-	20	80
113	"	F	14	ST(Business)	Pb	WC	1,2,14	Cq	-	20	20
114	"	M	2	Child(Farmer)	Pb	WC	1,6,9,10	Cq	-	40	160
115	"	F	29	C/S	Pb	WC	1,2	Cq	-	20	20
116	"	F	34	C/S	Pb	WC	1,2,3,8	Cq	-	40	40
117	"	F	30	Business	Pb	WC	2,4,6	Fan	-	20	20
118	"	M	6	Child(Business)	Pb	WC	2,5,6,10	-	-	20	20
119	"	M	26	(C/S)	W	P	1,2,3,8	Cq	-	40	20
120	"	F	30	C/S	Pb	WC	2,3,4,10,14	-	-	20	20
121	"	F	10	Child(C/S)	W	P	1,2,4,7	-	-	160	80
122	"	M	11	Child(Trader)	Pb	WC	1,2,4	Cq	-	20	20
123	"	F	37	Trader	Pb	WC	3,4,8,9	-	-	20	20
124	"	M	21	St(C/S)	W	P	1,3,8	-	-	160	80
125	"	F	25	Farmer	W	P	2,3,4,9	Cq	-	160	80
126	"	F	30	C/S	Pb	WC	4,9	-	-	20	20
127	"	F	34	ST(Business)	W	P	1,2,3,9,10	Cq	-	40	160
128	"	F	20	Trader	Pb	WC	1,2,5,6	Fan	-	20	20
129	"	M	20	ST(Business)	Pb	WC	1,4,6,11,13	Cq	-	40	20
130	"	F	40	Trader	Pb	WC	1,3,7	Cq	-	40	160

131	"	F	12	Child(Trader)	W	P	1,4	Cq	-	40	160
132	"	F	8	Child(C/S)	W	P	2,5,11	Mq	-	20	20
133	"	M	62	Farmer	Pb	WC	1,3,8	Fan	-	80	160
134	"	F	36	Trader	Pb	WC	2,9,10	Cq	-	20	80
135	"	F	31	Business	Pb	WC	1,4,8,9,10	-	-	40	160
136	"	F	37	C/S	W	P	2 3 4 5 6 8	-	-	20	20
137	"	F	37	C/S	Pb	WC	1 2 3 8	Cq	-	80	160
138	"	M	37	C/S	Pb	WC	4 8 1	Fan	-	20	20
139	"	F	25	C/S	Pb	WC	1 2 5 7 8 9 10	Cq	-	20	20
140	"	F	35	Trader	W	P	1 2 4 6 9	Fan	-	40	20
141	"	M	31	Trader	W	P	1 3 8	-	-	80	160
142	"	F	40	C/S	W	P	1 2 4 9 14	Cq	-	40	160
143	"	F	44	Trader	W	P	6 7	-	-	20	20
144	"	M	11	Child(c/s)	W	P	1 9 10	Mq	-	40	160
145	"	M	16	Trader	W	P	1 2 3 9 10 12	Cq	-	20	20
146	"	F	4	Child(C/S)	Pb	WC	1 5 8	Clph	-	40	80
147	"	M	13	St(Framing)	Pb	WC	1 3 4 9 10	-	-	20	20
148	"	F	6	Child(Trad)	W	P	4 8 9	-	-	20	20
149	"	M	50	Business	W	P	2 3 7 14	Cq	-	20	20
150	"	M	33	C/S	W	P	4 8 9 10	TC	-	20	20
151	"	F	4	Child(C/S)	Pb	WC	1 8 9	-	-	40	160
152	"	M	35	C/S	Pb	WC	6 7 8,9,10	-	-	20	20
153	"	M	14	St(Busin.)	W	P	1 2 8	Clph	-	40	40
154	"	F	16	St(Farming)	W	P	1 3 4	Cq	-	20	80
155	July	F	12	Child(Farming)	W	P	2 4 5 9	Cq	-	20	20
156	"	F	32	Trader	Pb	WC	1 2 3 6 7	Cq	-	40	160
157	"	M	3	Child(C/S)	Pb	WC	4 7 8 9 10	TC	-	20	80
158	"	M	45	C/S	Pb	WC	1 4 6 7	Cq	-	40	80
159	"	M	14	St(C/S)	W	P	1 7	Cq	-	20	20
160	"	F	39	Business	Pb	WC	1 4	Cq	-	40	40
161	"	M	15	Farmer	Pb	WC	1 2 11 14	Mq	-	20	20
162	"	F	56	St(C/S)	W	P	1 2 3 5 8	-	-	20	20
163	"	M	12	St(Busin.)	W	P	1 2 7	Cq	-	40	80
164	"	M	2	Child(Trader)	Pb	WC	1 8 9 10	-	-	40	160
165	"	F	6	Child(C/S)	Pb	WC	1 5 6 7	Cq	-	20	20
166	"	F	23	Business	Pb	WC	4	TC	-	20	20
167	"	M	18	Trader	W	P	4 6 7 8 9 10	-	-	80	20
168	"	M	49	Trader	Pb	P	1 2 4 5 11	Fan	-	160	80
169	"	M	56	C/S	W	P	3 4 11	-	-	20	80
170	"	M	30	C/S	Pb	WC	1 2 3 8	Cq	-	80	160
171	"	F	15	St(Trading)	Pb	WC	1 2 3 4 5 7 8	-	-	[b]640	320
172	"	F	41	C/S	W	P	2 4 5 6 11	-	-	20	20
173	"	M	38	Business	Pb	WC	1 3 4	-	-	40	160
174	"	F	3	Child(C/S)	Pb	WC	1 9	Clph	-	20	20
175	"	M	7	Child(Trading)	W	P	1 5 6 7	Cq	-	160	80
176	"	M	60	Business	Pb	WC	1 2 3	Fan	-	80	80

177	"	F	21	St(Farmer)	W	P	1 2 4	-	-	160	80
178	"	F	52	C/S	Pb	WC	2 5	Cq	-	20	20
179	"	M	35	C/S	Pb	WC	1 3 4 8 9	Mq	-	40	40
180	"	M	21	Business	Pb	WC	1 2 3 5	Cq	-	20	20
181	"	F	42	C/S	W	P	1 2 3 11 14	-	-	40	160
182	"	M	40	Business	W	P	1 2 3	Mq	-	20	20
183	"	M	16	Pastoralist	W	P	1 3 5 6 8	-	-	160	80
184	"	M	23	St(C/S)	W	W	1 2 4	Clph	-	20	20
185	"	F	57	Business	W	P	1 2 3	Cq	-	20	20
186	"	M	30	C/S	Pb	P	1 9 10	Cq	-	160	80
187	"	F	23	C/S	Pb	WC	1 2 4	-	+	20	80
188	"	M	49	C/S	W	P	1 3 4	-	-	160	80
189	"	F	5	Child C/S	Pb	WC	1 7 8 9	Clph	-	20	20
190	"	F	8½	Chiled(Trader)	Pb	WC	1 8 9 10	Cq	-	160	160
191	"	M	18	St C/S	Pb	WC	1 2 3 4 5 6 7 14	Cq	-	160	80
192	"	M	41	C/S	Pb	WC	2 3 9	Cq	-	20	80
193	"	F	17	(St)Trader	Pb	WC	1 2 3 14	-	-	80	160
194	"	M	16	St(Business)	Pb	WC	1 2 8 9	Fan	-	20	20
195	"	M	3	Child(Farmer)	Pb	WC	1 9 10	Cq	-	20	20
196	"	M	18	Trader	W	P	1 2 3 4 6 14	-	-	160	80
197	"	F	30	Business	Pb	WC	1	Cq	-	40	160
198	"	F	35	Trader	W	WC	1 3 6 8	Cq	-	40	80
199	"	M	25	Trader	Pb	WC	1 2 10	-	-	40	40
200	"	F	2	Child(Bus.)	Pb	WC	1 4 9 10	Mq	-	20	20
201	"	M	13	St C/S	W	P	1 2 4 8	-	-	80	160
202	"	F	42	C/S	Pb	WC	1 2 3	Cq	-	20	20
203	"	F	42	Business	W	P	1 2 4 10	Cq	-	40	160
204	"	F	24	Trader	W	P	2 3 6	Cq	-	20	20
205	"	M	4	Child(Bus.)	Pb	WC	1 4 6 8	Cq	-	20	20
206	"	F	20	St(Trader)	Pb	P	1 2 3 4 5 7 8	Cq	-	168	80
207	"	M	16	St(C/S)	Pb	WC	1 2 7	-	-	40	160
208	"	F	43	C/S	Pb	WC	1 3	-	-	80	160
209	"	M	51	C/S	Pb	WC	1 3 4	Fan	-	40	80
210	"	F	21	Trader	W	P	1 2 4 7 14	-	-	160	80
211	"	F	43	C/S	W	P	3 6 11	Cq	-	20	20
212	"	M	5	Child(Farmer)	W	P	1 5 8 10	Cq	-	160	80
213	"	F	7½	Child (C/S)	Pb	WC	1 4 9 10	Clph	-	40	80
214	"	F	25	Business	Pb	WC	2 3 7 10	Cq	-	20	20
215	"	M	18	Trader	Pb	P	1 3	Cq	-	160	80
216	"	F	36	Business	Pb	P	2 3 4 6 10	Cq	-	80	80
217	"	F	41	Business	PB	WC	1 2 3 14	Cq	-	40	160
218	"	M	10	Child (C/s)	W	P	7 8 9 10	TC	-	20	20
219	"	F	24	Trader	Pb	WC	1 2 7	Cq	-	40	160
220	"	M	31	C/S	Pb	WC	1 3 5	Cq	-	40	80
221	"	M	26	C/S	W	P	1 2 4	-	-	160	160
222	"	M	54	C/S	Pb	WC	1 3 4 5 9	-	-	40	160

223	"	M	25	Trader	Pb	WC	1 2 3	Fan	-	40	80
224	"	F	50	Trader	Pb	WC	1 2 4 9 14	Clph	-	40	80
225	"	M	60	Business	Pb	WC	1 2 4	Cq	-	20	20
226	"	M	21	Farmer	W	P	1 3 4 5	-	-	160	80
227	"	F	13	St(Trader)	Pb	P	1 2 7 14	Cq	-	160	80
228	"	F	1½	Child(Bus.)	Pb	WC	1 9	Cq	-	40	160
229	"	M	19	Farmer	Pb	WC	2 5	Cq	-	20	20
230	"	M	12	Child(Trader)	W	P	1 2 4	Cq	-	20	20
231	"	F	26	(Business)	Pb	WC	2 4 7	Cq	-	20	20
232	"	F	35	Trader	Pb	WC	1 2 4	Fan	-	40	40
233	"	M	45	C/S	Pb	WC	1 6 11	-	-	20	20
234	"	M	9	Child(C/S)	W	P	1 2 4 5	Cq	-	160	160
235	"	F	10	Child(Bus.)	Pb	WC	1 9 10	-	-	20	20
236	"	M	43	Farmer	Pb	WC	1 2 4 7 9 10	Cq	-	80	80
237	"	F	9	Child(C/S)	W	P	1 3 7 10	-	-	80	160
238	"	M	26	C/S	Pb	WC	1 2	Cq	-	40	40
239	"	M	20	St(Business)	Pb	WC	1 2 3 14	Cq	-	40	160
240	"	M	23	St(Trader)	W	P	4 5 6	-	-	20	20
241	"	M	24	Trader	W	P	1 2 4 6 7 11	Cq	-	160	160
242	"	F	63	Farmer	W	P	1 3 6	Cq	-	40	80
243	"	F	19	St(Farmer)	Pb	WC	1 2 4 5 6 7 8	Cq	-	80	20
244	"	M	16	St(Business)	Pb	P	1 2 7	Cq	-	160	160
245	"	F	18	St(Trader)	Pb	WC	4 9 10	-	-	20	20
246	"	M	20	St(Business)	Pb	WC	1	Fan	-	40	160
247	"	M	16	Trader	Pb	WC	2 3 5 6 8	Cq	-	40	80
248	"	M	13	St(Farmer)	Pb	WC	1 2 4 5 6 7 8	-	-	40	80
249	"	F	53	Business	W	WC	1 4 6 7 9	-	-	40	80
250	"	M	23	Business	W	P	1 2 5 6 7	Cq	+	80	160
251	Aug	F	25	Business	W	WC	1 7 9	Clph	-	20	20
252	"	F	12	St(Trader)	Pb	WC	9 10	-	-	20	20
253	"	M	30	C/S	Pb	WC	1 6 7 10	Cq	-	160	80
254	"	M	38	Business	Pb	WC	4	-	-	80	160
255	"	M	20	Trader	W	P	1	Cq	-	20	20
256	"	F	63	Farmer	Pb	WC	2 3 6 9	-	-	40	80
257	"	M	14	St (C/S)	Pb	Wc	1 5 6	Cq	-	20	20
258	"	M	24	C/S	Pb	WC	1 5 14	-	-	160	80
259	"	M	45	C/S	Pb	WC	1 2 7 8 10	Cq	-	160	160
260	"	M	30	Business	Pb	WC	1 2	-	-	40	160
261	"	F	24	Business	W	WC	1 2 5	Cq	-	20	20
262	"	M	30	Trader	Pb	WC	1 2 3 4	Cq	-	40	80
263	"	M	65	Farmer	Pb	WC	1 5 6	-	-	40	80
264	"	F	25	Trader	Pb	WC	1 2 3 4 6 8	Cq	-	640	320
265	"	M	7	Child(Farmer)	W	WC	1 8 9	-	-	40	80
266	"	M	30	Business	Pb	WC	1 2 14	Cq	-	20	20
267	"	F	18	C/S	W	WC	1 5 7	Clph	-	160	80
268	"	F	61	Trader	Pb	WC	1 3 5	-	-	40	160

269	"	M	13	St(Business)	Pb	WC	2 4	-	-	20	20
270	"	M	19	Farmer	W	P	1 2 4 6 7 10	Fan	-	320	160
271	"	F	10	Child(Trader)	Pb	WC	1	Mq	-	40	80
272	"	M	13	St(Business)	Pb	WC	1 2 3 5 6	-	-	40	160
273	"	F	52	Trader	Pb	WC	1 2 6 7 8	Mq	-	40	160
274	"	M	3	Child(Bus.)	Pb	WC	1 2 14	Hal	-	20	20
275	"	F	12	St(Farmer)	W	P	1 2 5 6	-	-	80	160
276	"	M	18	Trader	W	P	1 2 3 6 8 10	Cq	-	160	80
277	"	F	28	Business	W	P	4 6 9	-	-	20	20
278	"	M	22	Trader	Pb	WC	1 2	-	-	40	160
279	"	F	50	Trader	Pb	WC	1 2 7 8	Clph	-	20	20
280	"	M	34	C/S	Pb	WC	3 4	-	-	20	20
281	"	M	10	Business	W	P	1 5 9	Clph	-	80	40
282	"	M	25	Trader	Pb	WC	1 7 8 9 10	Cq	-	40	160
283	"	F	56	Business	W	P	1 2 3 6	Cq	-	20	20
284	"	M	45	C/S	Pb	WC	1 3 5	Cq	-	40	80
285	"	F	18	St(Bus.)	Pb	WC	1 6 9 14	Cq	-	20	20
286	"	F	28	Trader	W	P	1 2 4 6	Cq	-	160	160
287	"	M	38	C/S	Pb	WC	8 9 10	-	-	20	20
288	"	F	44	C/S	Pb	WC	1 2 3	Cq	-	20	20
289	"	M	36	C/S	W	P	4 11	-	-	20	20
290	"	M	20	St C/S	W	P	1 2 9	Fan	-	160	80
291	"	M	12	Child(Bus.)	Pb	WC	1 8	-	-	80	160
292	"	F	16	St(Trader)	Pb	WC	1	Cq	-	20	20
293	"	M	35	Trader	Pb	WC	2 5 10	Cq	-	20	20
294	"	M	38	Trader	Pb	WC	1 2 3	-	-	40	80
295	"	M	40	C/S	Pb	WC	1 2 6 8 11	Cq	-	40	80
296	"	M	16	Pastoralist	W	P	1 2 3 4 5 6 14	Cq	-	160	80
297	"	F	62	Business	Pb	WC	1 7 8	Mq	-	20	20
298	"	F	34	C/S	Pb	WC	1 7	Cq	-	20	20
299	"	M	14	St(Business)	W	P	1 13	Cq	-	20	20
300	"	M	19	Farmer	W	P	1 4 5 6 8 11	Cq	-	160	80
301	"	F	40	C/S	W	WC	2 3 4	Cq	-	20	20
302	"	M	45	Business	W	WC	1 2 3	-	-	40	160
303	"	F	30	Business	Pb	WC	1 5 6	Cq	-	40	80
304	"	M	18	Trader	W	P	1 2 3	-	-	80	160
305	"	M	35	Trader	Pb	WC	8 9	IC	-	20	20
306	"	M	35	C/S	Pb	WC	1 2 4 6 7 8 9 10	Cq	-	20	20
307	"	M	30	Farmer	W	P	1 4 3 7 8	Cq	-	320	160
308	"	F	36	C/S	W	P	2 3 4 11	Cq	-	20	20
309	"	M	17	St(Farmmer)	Pb	WC	1	Cq	-	40	160
310	"	f	2½	Child(Trader)	Pb	WC	1 5 6 7 8	-	-	20	20
311	"	F	17	St(Farmer)	W	P	1 2 6 8	Cq	-	160	160
312	"	M	28	C/S	W	P	1 2 4 5 14	-	-	160	80
313	"	F	4	Child(Trader)	W	P	1 9 10	Cq	-	40	80
314	"	M	13	St(Farmer)	W	P	1 2	-	-	80	160

315	"	F	63	Business	W	P	1 2 6			Fan	-	20	20
316	"	M	30	C/S	Pb	WC	1 4 5 7 11			Mq	-	160	80
317	"	M	26	C/S	Pb	WC	4 9			-	-	20	20
318	"	M	16	St(Trader)	W	WC	1			-	-	80	80
319	"	M	45	C/S	W	P	4 5 6 8			Clph	-	20	20
320	"	F	2	Child(Farmer)	Pb	WC	1 9 10 14			Cq	-	80	160
321	"	M	30	Trader	W	P	2 3 5			-	-	40	80
322	"	M	12	St(C/S)	Pb	WC	3 5 7			-	-	160	80
323	"	F	45	Business	W	P	1 2 3 14			Cq	-	20	20
324	"	F	28	Trader	W	P	2 3 4 9			-	-	160	160
325	"	M	19	St(Trader)	W	P	2 3 6			-	-	160	160
326	"	F	44	C/S	Pb	Wc	1 3 5			Hal	-	20	20
327	"	F	12	St(Farmer)	Pb	Wc	1 5 6			-	-	80	160
328	"	M	20	Trader	Pb	Wc	1			Cq	-	20	20
329	"	F	35	Trader	W	P	1 2 4 7			Cq	-	160	80
330	Sept	M	17	St(Business)	W	WC	1			Cq	-	20	20
331	"	M	17	Farmer	W	Wc	4 5 6 9			-	-	20	20
332	"	M	30	Trader	Pb	Wc	1 3 6			Hal	-	40	160
333	"	F	41	C/S	Pb	Wc	3 4 11			-	-	20	20
334	"	M	23	Trader	Pb	Wc	1 2			-	-	80	160
335	"	F	13	St(Farmer)	Pb	Wc	2 3 4			-	-	20	20
336	"	M	3	Child (Farmer)	Pb	Wc	1 5 10 14			Clph	-	40	80
337	"	M	29	C/S	W	Wc	1 2 3 5 6			Cq	-	160	80
338	"	F	38	Business	Pb	Wc	1 2 5			Cq	-	20	20
339	"	M	40	C/S	W	P	1 3 4 6			-	-	160	80
340	"	F	45	C/S	W	P	1 3			Cq	-	20	20
341	"	M	45	C/S	Pb	Wc	1 4			Far	-	20	20
342	"	F	32	Trader	Pb	Wc	1 2 6			Cq	-	40	160
343	"	M	41	Business	Pb	Wc	1 4 11 14			-	-	80	40
344	"	M	31	Business	W	P	1 2 6			Cq	-	80	160
345	"	F	42	C/S	Pb	Wc	1 3 8			Cq	-	20	20
346	"	F	15	St (Farmer)	W	P	1 2 14			Mq	-	40	80
347	"	M	20	Trader	W	P	1 2 4 7 8			Cq	-	160	160
348	"	M	11	Child (c/s)	W	P	7 9 10			-	-	160	80
349	"	F	44	c/s	W	P	1 3 6 14			Cq	-	80	160
350	"	M	46	Trader	W	P	4 8 10			-	-	20	20
351	"	M	25	Business	W	P	1 2			Cq	-	40	160
352	"	M	27	Business	W	P	1 2 3 4 6 14			Mq	-	40	160
353	"	M	34	Business	Pb	Wc	1 6			Hal	-	20	20
354	"	F	56	Business	Pb	Wc	1 2 3			Cq	-	80	160
355	"	M	31	Farmer	Pb	Wc	8 9			-	-	20	20
356	"	M	52	Trader	W	P	1 2 6			Amox	-	80	160
357	"	M	8	Business	Pb	Wc	1 4 7 10			Cq	-	20	20
358	"	F	8	Child (c/s)	Pb	Wc	4 8 9 14			-	-	20	20
359	"	F	6	Child(farmer)	Pb	Wc	1 4			Cq	-	40	160
360	"	M	63	Business	Pb	Wc	4 5 11			-	-	20	20

361	"	M	9	Child(farmer)	W	P	1 6 7 8			Clph	-	48	80
362	"	M	45	Farmer	Pb	Wc	2 4			Cq		20	20
363	"	F	65	Trader	W	P	1			Cq	-	20	20
364	"	M	49	Business	W	P	1			Cq	-	80	160
365	"	F	36	c/s	Pb	Wc	1 2 3 4 6			Cq	-	20	20
366	"	F	41	c/s	W	Wc	4 9			-	-	40	160
367	"	M	65	c/s	W	Wc	3 6 7			-	-	20	20
368	"	F	15	St (c/s)	W	P	1 4 7 8 11			Cq	-	160	80
369	"	M	43	Farmer	Pb	Wc	1 4 10 14			Cq	-	40	80
370	"	F	46	Business	Pb	Wc	4			-	-	20	20
371	"	M	9	Child(Trader)	W	P	1 2 8 9			-	-	160	80
372	"	M	55	Farmer	W	Wc	3 4			-	-	20	20
373	"	M	19	Trader	Pb	Wc	1 2 4			Hal	-	80	160
374	"	M	32	c/s	Pb	Wc	1 4			Mq	-	20	20
375	"	F	12	Child(Trader)	W	P	1 2 5 7			Cq	-	20	20
376	"	F	18	c/s	Pb	Wc	1 2 4 6			-	-	160	80
377	"	F	30	Farmer	Pb	P	1 2 5			Cq	-	160	80
378	"	F	5	Child(Farmer)	W	P	1 4 6 8			Hal	-	40	160
379	"	M	3	Child (Trader)	Pb	Wc	1 8 9			Mq	-	20	20
380	"	M	45	Farmer	Pb	Wc	1 3 5 8			Cq	-	20	20
381	"	F	14	St(Farmer)	Pb	Wc	1 2						
382	"	M	41	c/s	W	P	1 4 7 10			Cq	-	20	20
383	"	F	22	Trader	Pb	Wc	2 6 9 10			Tc	-	40	160
384	"	F	5	Child(Trader)	Pb	Wc	1 5 9			Cq	-	80	160
385	"	F	36	c/s	Pb	Wc	1			Cq	-	20	20
386	"	M	20	Trader	Pb	Wc	2 3 4 5			Cq	-	40	40
387	"	F	16	St(Trader)	W	P	1 2			Hal	-	20	20
388	"	M	8	Child(farmer)	W	P	4 5 7			-	-	80	160
389	"	M	31	Business	Pb	Wc	4 10			-	-	20	20
390	"	F	35	Trader	W	P	1 4 5 6			Cq	-	20	20
391	"	F	50	Business	Pb	Wc	1 2 3 4			Cq	-	20	20
392	"	F	25	Trader	W	P	1			Cq	-	40	160
393	"	M	31	Pastoralist	Pb	Wc	1 2 3 7			Clph	-	40	80
394	"	F	46	Trader	W	P	1 3			Cq	-	20	20
395	"	M	6	Child(Farmer)	W	P	1 4			Cq	-	80	160
396	"	F	64	Farmer	W	P	4 5 11			-	-	20	20
397	"	M	43	Farmer	Pb	Wc	3 4 11			Cq	-	20	20
398	"	M	18	Pastoralist	W	Wc	1 14			Clph	-	40	80
399	"	F	2	Child (Trader)	Pb	Wc	1 5 6 7 8			Hal	-	80	160
400	"	M	5	Child (Farmer)	Pb	Wc	1 7 14			Clph		20	20
401	"	F	31	c/s	Pb	WC	1 2 8			Cq	-	20	20
402	"	M	35	c/s	Pb	Wc	1			Cq	-	40	160
403	"	F	51	c/s	Pb	Wc	1 3 4			Cq	-	20	20
404	"	F	24	Trader	Pb	Wc	8 9 10			-	-	20	20
405	"	M	21	Pastoralist	W	Wc	1 2 4			Mq	-	40	160
406	"	F	36	c/s	Pb	Wc	1 6 7 8 9 10 14			Amox	-	40	80

407	"	F	2½	Child(Trader)	W	P	1		Cq	-	80	160
408	"	F	55	Trader	W	P	4 5		-	-	20	20
409	"	F	42	Business	W	P	1 2 5 6		Cq	-	20	20
410	"	F	8	Child(Farmer)	W	P	1 2 8		-	-	160	80
411	"	F	49	Trader	Pb	Wc	1 2 3		Mq	-	80	160
412	"	M	24	C/S	Pb	Wc	1 9 14		Cq	-	40	80
413	"	F	5	Child(Farmer)	Pb	Wc	1 5 8		Fan	-	40	160
414	"	F	16	St(Farmer)	W	P	4 5		-	-	20	20
415	"	M	23	Trader	W	Wc	1 3 7		Clph	-	40	160
416	"	M	21	Trader	Pb	Wc	8 9		-	-	20	20
417	"	F	33	Business	W	P	6 8 10		-	-	40	160
418	"	M	39	Farmer	W	P	4 6 11 14		-	-	20	20
419	"	F	25	c/s	W	Wc	1 4 5		Cq	-	80	20
420	"	F	3½	Child(Farmer)	Wp	Wc	1 9		Cq	-	80	160
421	"	F	9	Child(Trader)	Pb	Wc	1 5 6 8 14		Cq	-	80	160
422	"	F	42	c/s	Pb	Wc	6 9		-	-	20	20
423	"	M	32	Trader	W	P	6 9 10		-	-	20	20
424	"	M	50	Farmer	W	P	1 2 4 8		Cq	-	160	80
425	"	M	19	Pastoralist	W	P	1 2 4 5 6 8		Cq	-	160	80
426	"	F	6	Child(Farmer)	Pb	Wc	1 8 9 10		Mq	-	20	20
427	"	F	31	Business	Pb	Wc	1 5 11		Cq	-	40	160
428	"	F	43	Business	Pb	Wc	1 4		Cq	-	20	20
429	"	F	3	Child(Farmer)	W	P	1 5 14		-	-	80	160
430	"	F	5	Child(Trader)	W	P	1 8 14		Cq	-	40	80
431	"	F	43	c/s	Pb	Wc	1 2 4 6 8		Cq	-	80	160
432	"	M	56	Farmer	Pb	Wc	1 8 9		Clph	-	40	80
433	"	F	10	Child(Farmer)	Pb	Wc	1 2 5 9		Cq		20	20
434	"	F	65	Trader	Pb	Wc	1 2 6		-	-	80	160
435	"	M	59	Trader	Pb	Wc	1 2 6 7		Cq	-	20	20
436	"	M	22	St(Farmer)	Pb	Wc	1 3 8 9 14		Cq	-	20	20
437	"	F	24	Trader	Pb	Wc	2 4		-	-	80	160
438	"	M	17	St(Farmer)	W	P	1 4 6 8 9		-	-	20	20
439	"	F	8	Child(Farmer)	Pb	Wc	1 4		Amox	-	40	160
440	"	M	6	Child(Farmer)	W	P	1 6		-	-	80	160
441	"	F	4	Child(Farmer)	W	P	1		Fan	-	20	20
442	"	M	19	Trader	Pb	Wc	1 3 5 7 14		Fan	-	20	20
443	"	M	9	Child(Trader)	Pb	Wc	1 2 4		Cq	-	20	20
444	Oct.	M	14	St(Farmer)	Pb	Wc	2 4		-	-	40	80
445	"	F	27	Trader	Pb	Wc	1 2 5		Cq	-	40	80
446	"	F	21	Trader	W	P	1 3		Cq	-	80	160
447	"	F	36	Farmer	Pb	Wc	1 2 4		Cq	-	80	160
448	"	M	16	St(Farmer)	W	Wc	3 7 8		-	-	80	160
449	"	M	11	Child(Trader)	W	Wc	2 3 8 9 10		-	-	80	160
450	"	F	10	Child(Farmer)	Pb	Wc	1 2 56		Cq	-	20	20
451	"	M	25	Pastolarist	Pb	Wc	1 2 4 14		-	-	20	20
452	"	M	48	c/s	Pb	Wc	1		Cq	-	40	160

453	"	F	64	Farmer	W	P	1 2 7	-	-	160	80
454	"	M	31	c/s	W	P	1 3 4	Cq	-	40	160
455	"	F	4	Child(Farmer)	W	P	14 10 11	-	-	20	20
456	"	F	9	Child(Trader)	Pb	Wc	1 8	Mq	-	80	160
457	"	M	6	Child (Farmer)	W	P	1 14	Cq	-	40	160
458	"	F	10	Child (c/s)	W	Wc	1 89	Cq	-	160	80
459	"	F	21	Trader	Wp	Wc	2 4	-	-	20	20
460	"	M	45	c/	W	Wc	1 3 4 7	Clph	-	40	160
461	"	M	13	Pastoralist	W	P	1 2 6	Fan	-	80	160
462	"	F	27	Trader	Pb	Wc	1 2	-	-	80	160
463	"	F	31	c/s	Pb	Wc	1 2 3 5	Cq	-	40	80
464	"	F	52	c/s	Pb	Wc	3 10	-	-	20	20
465	"	M	34	Farmer	W	Wc	2 4 7	-	-	160	80
466	"	M	36	Farmer	Pb	Wc	1	Cq	-	160	80
467	"	F	45	Business	W	P	2 4	Cq	-	80	160
468	"	F	12	St(Farmer)	W	P	1	Cq	-	40	160
469	"	F	47	Trader	W	P	1 6	Fan	-	20	20
470	"	F	60	Business	Pb	Wc	2 3 7	-	-	40	160
471	"	F	16	St(c/s)	Pb	Wc	1 4 7 14	Cq	-	160	160
472	"	F	24	Business	Pb	Wc	1 4 5	Fan	-	20	20
473	"	F	36	c/s	Pb	Wc	1 14	-	-	80	160
474	"	M	19	Trader	W	Wc	1 2 3 6	Cq	-	80	160
475	"	F	31	c/s	Pb	Wc	1 3	Cq	-	40	160
476	"	M	40	c/s	W	P	1 4	Cq	-	20	20
477	"	F	65	Trader	W	P	1 2 3	-	-	20	20
478	"	M	65	Farmer	W	P	1 2 3 4	Clph		160	160
479	"	F	21	c/s	W	P	1 3 4 6 8	Cq	-	20	20
480	"	M	27	c/s	Pb	Wc	1 4	-	-	20	20
481	"	M	36	c/s	Pb	Wc	2 3 11	-	-	20	20
482	"	F	45	Business	W	Wc	1 2 3 4 9 10	Cq	-	20	80
483	"	F	56	Trader	Pb	Wc	1 9	Cq	-	20	20
484	"	F	19	St(Farmer)	W	P	1 5	Cq	-	20	20
485	"	F	43	Business	W	Wc	3 4 6 7	Clph	-	40	160
486	"	F	10	Child(Farmer)	Pb	Wc	1 4 5 8	Cq	-	20	20
487	"	F	8	Child(Farmer)	W	Wc	1 2 5 6 8 10	Cq	-	20	20
488	"	F	6	Child(Business)	W	P	1 4 6 7 9	-	-	20	20
489	"	M	42	Farmer	W	P	3 4	-	-	20	20
490	"	F	41	Farmer	W	P	2 5 7	-	-	20	20
491	"	M	17	Pastoralist	W	Wc	1 2 3 11	Cq	-	20	20
492	"	F	21	c/s	Pb	Wc	8 9	-	-	20	20
493	"	M	41	c/s	Pb	Wc	1 3 4 8	Cq	-	160	80
494	"	M	13	St(Farmer)	Pb	Wc	2 3 5 7	Cq	-	20	20
495	"	F	24	St(Farmer)	W	P	1 2 6 7	Cq	-	40	160
496	"	M	26	Farmer	S	P	2 5 14	-	-	20	20
497	"	M	16	St(Farmer)	W	P	1 4 5 8 9 10	Cq	-	40	160
498	"	M	38	Farmer	Pb	Wc	1 2 3	Cq	-	20	20

499	"	F	52	Business	Pb	Wc 1 4	Cq -	20	20
500	"	M	15	St(Farmer)	W	P 1 4 5 8 10	Cq -	20	20

Keys

*Occupational background of child or student in parenthesis

M = Male
F = Female
Cs = Civil Servant
St = Student
Cq = Chloroquine
Mq = Melaquine

P = Pit Latrine
W = Well
Pb = Pipeborne
Wc = Water Closet
Cq = Fancidar
Hal = Halfan
Tc = Tetracycline

Clph = Chlormphenicol
Amox = Amoxicillin
1 = Fever
2 = Headache
4 = abdominal pains
5 = Insomnia
6 = Anorexia
7 = Weight loss
8 = Weakness
9 = Diarrhoea

10=Vomiting
11=Constipation
12=Rash
13=Mental Confusion
14=Cough
15=Coma
a 20 represents the antibody titres less than or equal to 20 (≤ 20)
b 640 represents ≥640.

APPENDIX II

Zone diameter interpretive Standard (mm)

Antibiotic	Code	Resis	Inter	Suscep
1. Chloramphenicol	C-30	≤12	13-17	≥18
2. Flucloxacillin	Fu-5	≤12	13-15	≥16
3. Gentamicin	GM-10	≤12	13-14	≥15
4. Cefuroxime	CXM-30	≤14	15-17	≥18
5. Ceftaxidime	CAZ-30	≤14	15-17	≥18
6. Amoxicillin (20µg)/ Clavulanic Acid (10µg) (Augmentin)	AMC-30	≤13	14-17	≥18
7. Azithromycin	AZM-15	≤13	14-17	≥18
8. Ampicillin 10µg/ Sulbactam (10µg)	SAM-20	≤11	12-14	≥15
9. Trimethoprim (1.25µg/ Sulfamethoxazole (23.75µg)	SXT-25	≤10	11-15	≥16