PREVELENCE OF SALMONELLA TYPHI AND ANAEMIA (PCV) AMONG PATIENTS ATTENDING UNIVERSITY COLLEGE HOSPITAL IBADAN, OYO STATE

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IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF NATIONAL DIPLOMA (ND) IN SCIENCE LABORATORY TECHNOLOGY.

SEPTEMBER, 2016.

CERTIFICATION

This is to certify that this project was carried out by **ODUSANYA, Oluwatosin R (Matric No: 14/06/3107)** of department of science laboratory technology Abraham Adesanya polytechnics, Ijebu Igbo, Ogun State under my Supervision.

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Supervisor's Name

18-11-16

Signature

DEDICATION

This research study is dedicated to Almighty God for His infinite mercy, support, guidance, blessing, protection over me throughout the period of my study.

ACKNOWLEDGEMENTS

I give thanks and adoration to ALMIHGTY GOD for His guidance, protection and infinite mercy upon my life throughout the period of my study.

My hearty gratitude goes to my dear husband and my parents for their support morally, financially, spiritually and materially throughout the course of my academic pursuit. May God reward you abundantly and keep you (Amen).

Special thanks go to my supervisor and all members of staff of the Department for the effort they made in making this project a successful one. Thank you very much.

ABSTRACT

This study examined the prevalence of *Salmonella typhi* and aneamia (PCV) among patients attending university college hospital Ibadan, Oyo state, Nigeria. *Salmonella typhi* causes typhoid fever which is an endemic disease in the tropic and sub-tropic and has become a major public health problem in developing countries of the world. Two hundred participants were used for the study. Blood samples were collected from 100 male and 100 female patients for this study. Findings revealed that 68 % were positive while 32 % were negative among males and 46% were positive while 54% were tested negative among females.

Based on this finding, adequate and improved sanitation, sewage systems, proper hygiene and better means of isolating the organism from culture should be adopted by the society and health care delivery system.

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CHAPTER ONE

1.1 INTRODUCTION

In 2009, over 40,000 cases of *Salmonella* (13.6 cases per 100,000 persons) were reported to the Centers for Disease Control and Prevention (CDC) by public health laboratories across the nation, representing a decrease of approximately 15% from the previous year, but a 4.2% increase since 1996. Overall, the incidence of *Salmonella* in the United States has not significantly changed since 1996 (Philip, 2000). Only a small proportion of all *Salmonella* infections are diagnosed and reported to health departments. It is estimated that for every reported case, there are approximately 38.6 undiagnosed infections. The CDC estimates that 1.4 million cases, 15,000 hospitalizations, and 400 deaths are caused by *Salmonella* infections in the U.S. every year (WHO, 2008).

Salmonella can be grouped into more than 2,400 serotypes. The two most common serotypes in the U.S. are *S.* Typhimurium and *S.* Enteritidis. *S.* Typhi, the serotype that causes typhoid fever, is uncommon in the U.S. (Jegathesan, 1984). But, globally, typhoid fever continues to be a significant problem, with an estimated 12-33 million cases occurring annually. Moreover, outbreaks in developing countries have a high death-rate, especially when caused by strains of the bacterium that are resistant to antibiotic treatment (Philip, 2000).

Typhoid fever (enteric fever) caused by Salmonella typhi is an endemic disease in the tropic and sub-tropic and has become a major public health problem in developing countries of the world with an estimated annual incidence of 540 per 100,000. The annual incidence of typhoid is estimated to be about 17 million cases worldwide (WHO, 2008).

Salmonella are found in the intestinal tract of wild and domesticated animals and humans. Some serotypes of *Salmonella*, such as *S.* Typhi and *S.* Paratyphi are only found in humans. For ease of discussion, it is generally useful to group *Salmonellae* into two broad categories: typhoidal,

which includes *S.* Typhi and *S.* Paratyphi, and non-typhoidal, which includes all other serotypes. Typhoid and paratyphoid fevers are infections caused by bacteria, which are transmitted from faeces to ingestion. Clean water, hygiene and good sanitation prevent the spread of typhoid and paratyphoid (Philip, 2000). Contaminated water is one of the pathways of transmission of the disease (WHO, 2008). Typhoid and paratyphoid fevers are caused by the bacteria *Salmonella typhi* and *Salmonella paratyphi*, respectively.

Anemia makes important contributions to disease burden in most low- and middle-income countries. The burden of anemia remains highest in sub-Saharan Africa, with Nigeria also heavily affected. The global anemia prevalence in 2010 was 32.9%, resulting in 68.4 million years lived with disability (YLD). The results emphasize the important contribution made by anemia to the overall global burden of disease and should help focus attention and resources toward this problem (Kassebaum et al., 2014)

Anemia makes important contributions to disease burden in most low- and middle-income countries. The burden of anemia remains highest in sub-Saharan Africa, with South Asia also heavily affected. Several previous efforts have summarized the global prevalence and impact of anemia. In 1985, the World Health Organization (WHO) estimated that about 30% of the world population was anemic (World Health Organization, 2008). In 1992, the WHO estimated that 37% of all women were anemic. Kassebaum et al (2014) have furthered these analyses to provide detailed estimates of the prevalence and epidemiology of anemia, its impacts on global health, and its key determinants, stratified by age and sex, and compared these between 1990 and 2010.

Prevalence of typhoid fever caused by Salmonella typhi in Nigeria in the contemporary time has become one issue that cast a gloomy shadow to the entire Nigerian society especially among adults. Typhoid and paratyphoid fevers are caused by the bacteria Salmonella typhi and Salmonella paratyphi, respectively. Typhoid fever (enteric fever) caused by Salmonella typhi is

an endemic disease in the tropic and sub-tropic and has become a major public health problem in developing countries of the world with an estimated annual incidence of 540 per 100,000. The annual incidence of typhoid is estimated to be about 17 million cases worldwide (WHO, 2008). It is often encountered in tropical countries including Nigeria where they constitute serious sources of morbidities and mortalities (Ibekwe et al., 2008). Anemia makes important contributions to disease burden in most low- and middle-income countries. The burden of anemia remains highest in sub-Saharan Africa, with Nigeria also heavily affected. To this end and judging from the problems outlined earlier, this research aims at investigating the prevalence of Salmonella typhi and Aneamia among patients in university college hospital Ibadan, Oyo state, Nigeria.

1.2. Aims and Objectives of the study

The objective of this study is to investigate the prevalence of Salmonella typhi and Aneamia among patients in university college hospital Ibadan, Oyo state, to enlighten them on ways by which it could be prevented and controlled among them and to provide useful information to the general public, university college hospital Ibadan and public health department on prevalence of Salmonella typhi and Aneamia.

CHAPTER TWO

2.0 LITERATURE REVIEW

Salmonella typhi is Gram-negative bacteria, which are motile, though non-flagellate variants, occur. Capsules are not formed. They are intestinal pathogens, which comprises of a species *Salmonella typhi*, which causes an enteric fever known as typhoid fever (Philip, 2000). It is pathogenic to both man and mammals with associable inflammatory reaction in the intestinal tract.

Typhoid fever is among the water-borne infections (Singh and Mcfeters, 1992) characteristic of environments with poor sanitation and hygiene. It is a health problem that has been associated with development (Jegathesan, 1984). Human infection with *Samonella* is mainly by the oral route through ingestion of faecal contaminated food and water, unclean hands, flies and meat from infected animals. Typhoid and paratyphoid germs are passed in the faeces and urine of infected people. People become infected after eating food or drinking beverages that have been handled by a person who is infected or by drinking water that has been contaminated by sewage containing the bacteria. Once the bacteria enter the person's body they multiply and spread from the intestines, into the bloodstream (WHO, 2008). Even after recovery from typhoid or paratyphoid, a small number of individuals (called carriers) continue to carry the bacteria. These people can be a source of infection for others. The transmission of typhoid and paratyphoid in less-industrialized countries may be due to contaminated food or water. In some countries, shellfish taken from sewage-contaminated beds is an important route of infection. Where water quality is high, and chlorinated water piped into the house is widely available, transmission is more likely to occur via food contaminated by carriers handling food (WHO, 2008). *Salmonella typhi* have somatic antigens and glycolipid microcapsule the vi or virulence antigen. Phage typing can distinguish different strains of the organism. Enteric fever caused by *Salmonella typhi*

is often encountered in tropical countries including Nigeria where they constitute serious sources of morbidities and mortalities (Baver, 1995). It is a major public health problem in the developing countries of the world with an estimated annual incidence of 540 per 100,000 (Ibekwe *et al.*, 2008).

Salmonella are divided into distinct serologic groups (A through E) on the basis of their somatic O antigens. While all group D organisms, such as *S. typhi* possess O antigen 9, about 60 of the 78 groups D serotypes including *S. typhi* also have O antigen 12 (Hook, 1985). Thus, infection by any of the group D serotypes can produce antibodies that can react with the O antigen used in the Widal reaction (Olopoenia *et al.*, 2000). Also, since all groups A and B organisms possess O antigen 12, cross-reactions with O antibody of group D serotype can occur with any of the group A and B serotype O antigens. Depending on the relative quality and quantity of antigenicity of the O antigens 9 and 12 contained in other common non-typhoidal *Salmonella serotypes*, cross-reaction may occur frequently enough to lessen considerably the diagnostic specificity of the Widal agglutination reaction (Olopoenia *et al.*, 2000).

In endemic areas, most individuals are careers. Thus, 35.9% of such apparently healthy persons have been detected with normal antibody titres of up to 1:40 and 1:80 for O and H *Salmonella antigens* (Tanyigna *et al.*, 1999) and the levels reflected severity of infection with *Salmonella*. Even though the associable mortality rate is very low, the high prevalence of salmonellosis has caused major economic and health impacts. As such, vaccines have been developed against strains of *Salomonella* (Myron *et al.*, 1976).

Based on the immunology of *Salmonella* infection, serological diagnostic tests relying on *Salmonella antigens* as a tentative evidence of salmonellosis have been developed, notably, the Widal agglutination test (Outi *et al.*, 1989). Agglutination is a classic serologic reaction that

results in clumping of a cell suspension by a specific antibody, directed against a specific antigen. Such tests have been widely used for detection of antibodies against various disease-producing microorganisms in serum for a long time (Olopoenia *et al.*, 2000).

The Widal agglutination test, developed by Widal in 1896 to aid in the diagnosis of typhoid fever, utilizes a suspension of killed *Salmonella typhi* as antigen, to detect typhoid fever in serum from suspected S typhi-infected patients who present with febrile illness. The value and clinical application of the Widal test in developed countries has diminished considerably in recent years (Washington and Henry, 1991) and a large number of antigenically related determinants of both typhoid and non-typhoid *Salmonella* organisms are now recognized (Olopoenia *et al.*, 2000).

The Widal test is a presumptive serological test for Enteric fever or Undulant fever. In case of *Salmonella* infections, it is a demonstration of agglutinating antibodies against antigens O-somatic and H-flagellar in the blood. Two types of agglutination techniques are available: the slide test and the tube test. The slide test is rapid and is used as a screening procedure. Using commercially available antigens of *S. typhi*, a drop of the suspended antigen is added to an equal amount of previously prepared serum. An initial positive screening test requires the determination of the strength of the antibody. This is done by adding together equal amounts of antigen suspension and serially diluted serum from the suspected patient. Agglutinations are visualised as clumps.

Weakly reactive agglutinations may require an adequate light source for proper visualisation, while strongly reactive agglutinations are easily seen. The result of the tests is scored from 0 to 4+, i.e., 0 (no agglutination), 1+ (25% agglutination), 2+ (50% agglutination), 3+ (75% agglutination) or 4+ (100% agglutination). The smallest quantity of serum that exhibits a 2+ or 50% agglutination is considered the end-point of serum activity or titre (Olopoenia *et al.*, 2000).

The tube agglutination test requires much more technical work than the rapid slide test, and is a macroscopic test. It also serves as a means of confirming the results of the slide test. A mixture of suspended antigen and antibody is incubated for up to 20 h at 37°C in a water bath. Agglutinations are visualised in the form of pellets, clumped together at the bottom of the test tube. Results are scored from 0 to 4+ positive agglutination as described above for the slide test. The tube test is useful to clarify erratic or equivocal agglutination reactions obtained by the more rapid slide test (Olopoenia *et al.*, 2000). Widal agglutination was introduced as a serologic technique to aid in diagnosis of typhoid fever. The test was based on demonstrating the presence of agglutinin (antibody) in the serum of an infected patient, against the H (flagellar) and O (somatic) antigens of *Salmonella typhi* (Olopoenia *et al.*, 2000). It is not a very accurate method, since patients are often exposed to other bacteria (e.g. *Salmonella enteritidis, Salmonella typhimurium*) in this species that induce crossreactivity; many people have antibodies against these enteric pathogens, which also react with the antigens in the Widal test, causing a false-positive result. Test results need to be interpreted carefully in the light of past history of enteric fever, typhoid vaccination, and general level of antibodies in the populations in endemic areas of the world.

Other means of diagnosing *Salmonella typhi* (and paratyphi) include cultures of blood, urine and feces. The organism also produces H2S from thiosulfate. Often 2-marceptoethanol is added. This agent binds to the IgM class of antibodies, so if a decrease in the titer is seen after using this agent, it means that it's IgM that's high but not IgG. This differentation of antibody classes is important; as it allows for the distinction of a recent (IgM) from an old infection (IgE). Typhidot is the other test used to ascertain the diagnosis of typhoid fever. As with all serological tests, the rise in antibody levels needed to make the diagnosis takes 7-14 days, which limits their use (Olopoenia *et al.*, 2000). While the definitive diagnosis of typhoid fever depends on the isolation

of *S. typhi* from blood, stools, urine or other body fluids (Gilman *et al.*, 1975 and Manson-Bahr), the role of the Widal test had been to increase the index of suspicion for the presence of typhoid fever by demonstrating a positive agglutination during the acute and convalescent period of infection with evidence of a four-fold rise of antibody titre (Somerville *et al.*, 1981).

In developed countries, the use of Widal agglutination as a laboratory tool to aid in the diagnosis of typhoid fever during the acute phase of the illness has largely been abandoned (Washington and Henry, 1991), as the need for such a test is minimal, especially in view of the low prevalence of typhoid fever. In addition, adequate and improved sanitation, sewage systems, proper hygiene and better means of isolating the organism from culture are available (Olopoenia *et al.*, 2000). Unfortunately, in some developing countries, the situation is quite different, and the Widal test appears to be the only laboratory means employed in the diagnosis of typhoid fever among suspected patients (Olopoenia *et al.*, 2000). As the test suffers from serious cross-reactivity with other infectious agents, it may produce false-positive results, leading to an over-diagnosis of typhoid fever. Reynolds *et al.* (1970) concluded that diagnosis of typhoid fever based on serology (Widal agglutination) alone is frequently inaccurate. Concomitant with this increase in diagnosis is the abuse of the first-line drug of choice (chloramphenicol), which has led to the selection of resistant strains of *S. typhi* (Olopoenia *et al.*, 2000).

Over 100 years since its introduction as a serologic means of detecting the presence of typhoid fever, the Widal test continues to be plagued with controversies involving the quality of the antigens used and interpretation of the result, particularly in endemic areas.

The significance of the Widal agglutination test in the diagnosis of typhoid fever has been reviewed by Olopoenia *et al.* (2000). Georges-Fernand Widal test if TO antigen more than 1.160

in active infection widal test if TH antigen more than 1.160 in past infection or in immunized (Olopoenia *et al.*, 2000). Areas of concern with clinical and laboratory significance of Widal test has been reviewed in the past and this include: the techniques of test performance, interpretation of results, limitation of the value of the test results in endemic typhoid areas, the quality of the antigens used, and alternative diagnostic tests. Similarly, the reliability of serologic test in solely diagnosing typhoid fever has suffered doubt. It has been reported to remain positive, months after an effective therapy of the infection (Outi *et al.*, 1989) such that a positive test may not necessarily indicate active infection, making the test be of relevance in diagnosing postinfection complications. Also, the quality of *Salmonella antigens* and interpretation of results, specifically in the Widal agglutination test have been identified as areas of controversy (Olopoenia *et al.*, 2000), hence, the suitability of stool culture alongside serologic test in diagnosing active infection (Adeleke *et al.*, 2006).

In Nigeria, the Widal agglutination test is about the sole laboratory diagnostic tool employed to buttress clinical diagnosis of enteric fever for the purpose of directing therapeutic measures specifically against this malady (Ibekwe *et al.*, 2008). As is generally known, the results of this serological test only become reliable if at least two properly staggered tests show about four-fold rise in antibody levels (Gilles, 1975). While performance of the test may require some detailed technical work, interpreting the test result is the more arduous task (Olopoenia *et al.*, 2000). Since the ultimate goal of the test is antigen-antibody complex reaction, cross-reactions are encountered when antibody produced by nontyphoidal antigens reacts with typhoid-specific antigens.

Several other diseases caused by non-Salmonella organisms (malaria, dengue, miliary tuberculosis, endocarditis, chronic liver disease, brucellosis, etc) have been shown to exhibit this cross-reactivity in typhoid endemic regions, and these cross-reactions increase the error rate of

the result of the Widal test (Olopoenia *et al.*, 2000). However, the scientific turism remains that only the bacteriological isolation of enteric fever bacteria from the patients' blood, faeces or urine constitute unequivocal evidence of the infection (Opera and Nweke, 1991; Ibekwe *et al.*, 2008).

The use of the Widal test to diagnose typhoid fever should therefore be limited to situations in w hich there is no other confirmatory supportive test, such as positive culture, available (Olopoenia *et al.*, 2000). Similarities between typhoidal and non-typhoidal *Salmonella antigens* mean that a serological method of diagnosis is the least accurate for typhoid fever. Due to the inexperience of some clinicians in typhoid endemic countries, many cases of pyrexia of unknown origin receive the diagnosis of typhoid fever, based upon a false-positive Widal test result rather than a positive culture of *S. typhi* (Olopoenia *et al.*, 2000).

In Nigeria, the harsh economic climate has encouraged a cancerous rate of household production of various food products with the attendant risk to public health (Adeleke *et al.*, 2006). As a matter of fact, the unreported cases of water-borne infections, particularly typhoid fever, have been more than those reported to hospitals for treating 7 female patients (77 samples) of different age groups (Adeleke *et al.*, 2006).

In the issue of *Blood*, Kassebaum et al (2014) estimate that the global anemia prevalence in 2010 was 32.9%, resulting in 68.4 million years lived with disability (YLD). The results emphasize the important contribution made by anemia to the overall global burden of disease and should help focus attention and resources toward this problem.

Anemia makes important contributions to disease burden in most low- and middle-income countries. The burden of anemia remains highest in sub-Saharan Africa, with South Asia also heavily affected. Several previous efforts have summarized the global prevalence and impact of

anemia. In 1985, the World Health Organization (WHO) estimated that about 30% of the world population was anemic (World Health Organization, 2008). In 1992, the WHO estimated that 37% of all women were anemic. Kassebaum et al (2014) have furthered these analyses to provide detailed estimates of the prevalence and epidemiology of anemia, its impacts on global health, and its key determinants, stratified by age and sex, and compared these between 1990 and 2010. To achieve this, the authors used data from 409 data sets from the Demographic and Health Surveys (which are national, weighted surveys of health status, with anemia measured by capillary hemoglobin) and WHO databases that contain data from national and subnational epidemiologic surveys. The 17 specific causes that contribute to anemia were then apportioned by using data from the Global Burden of Diseases, Injuries and Risk Factors 2010 (GBD 2010) study, with residual prevalence after attribution assigned to other causes, including iron deficiency anemia (WHO, 2008).

Next, the authors estimated the impact of anemia on global health (disease burden). To appreciate disease burden estimates, it is worth looking under the hood at their calculation. The first concept is the disability weight (DW), a value representing the severity of health loss associated with a clinical condition. Previous DW estimations were criticized for being determined by expert committees; thus, for GBD 2010, DWs were developed by opinion from the general public through a comprehensive international study (30 230 participants) comprising household surveys in Bangladesh, Indonesia, Peru, Tanzania, and the United States, together with an open-access Internet survey (Mathers et al., 2002).

Participants were given descriptions of 2 hypothetical patients with particular health states and asked which individual they considered healthier. The Internet survey also asked respondents to compare the value of various life-saving or disease prevention programs. Thus, 220 health

conditions (including mild, moderate, and severe anemia) were ranked, and DWs were assigned (with 0 for the mildest and 1 for the equivalent of death). Mild anemia was the third mildest of all conditions (DW of 0.005, more serious only than "mild impairment in distance vision" and "treated, long-term fractures"), whereas moderate and severe anemia had DWs of 0.058 (similar to "moderate hearing loss with ringing") and 0.164 (similar to "amputation of one leg, long term"). The DWs for mild, moderate, and severe anemia were each higher in GBD 2010 than in the previous report. The YLD, an estimate of the total number of years lived in less-than-ideal health due to a condition, is calculated by multiplying its prevalence by its DW, and this figure estimates the burden of each disease (Vos, et al., 2010)

By using this approach, the authors found that anemia accounts for 8.8% of the world's YLD, but that the prevalence of anemia worldwide has decreased from 1990 to 2010, with most of the improvement coming from a genuine reduction in the conditions that cause anemia. This raises an important research question: What combination of improvements in proximal and distal determinants of anemia have effected this change? Children younger than age 5 years still have the highest prevalence of, and the most severe, anemia and had rising prevalence in contrast to the overall trend and findings from other reports. South Asia accounted for 37.5% of the global anemia burden, whereas sub-Saharan Africa contributed 23.9%.

The main causes of anemia worldwide were attributable to 3 syndromes: iron deficiency (iron deficiency anemia, hookworm, and schistosomiasis), hemoglobinopathy (sickle cell disorders and thalassemia), and malaria. Coexistence of iron deficiency and malaria as top causes of anemia in sub-Saharan Africa highlight the paradox for anemia control in such regions, insofar as iron supplementation might increase malaria risk. Another important finding of this study is the increasing contribution from chronic kidney disease, which increased over the 2 decades of

the study and is the chief cause of anemia in the 80+ age group. WHO and the US Centers for Disease Control and Prevention define anemia in children younger than age 5 years as hemoglobin <110g/L. However, the authors defined anemia in this age group as hemoglobin <120g/L, and they acknowledge that this has inflated estimates of the prevalence of anemia in children in this age group by 16.3% in males and 18.1% in females, on average, although by even more in some cases. This should be borne in mind when interpreting estimates of prevalence and burden of anemia in this age group (WHO, 2008).

Although founded in data, this report presents modeling-based estimates of the determinants of anemia and does not replace the need for field epidemiology to measure the contributions to anemia of factors such as iron, folate, vitamin B_{12}, and vitamin A deficiencies; hemoglobinopathy; malaria; inflammation; and other causes in specific settings; such studies are rare outside (and even within) developed nations. Nevertheless, Kassebaum et al have developed a valuable resource that confirms the significance of anemia within the overall context of global health, provides the first global estimates of its causes, and thus will be essential in guiding future control strategies (Kassebaum et al., 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

An experimental survey design was used in determining the prevalence of salmonella typhi and aneamia among patients in university college hospital Ibadan, Oyo state.

3.2 Study Area

This study was conducted in university college hospital Ibadan, Oyo state. Ibadan, Oyo State is one of the parts of the Yoruba cultural region of southwestern Nigeria. The city was founded in the 19th century when several small towns united for purposes of defense during the wars brought about by the Oyo Empire. This city has experienced both population and economic growth since the 1950s due to its position between the cities of south west. University college hospital Ibadan is a government -owned federal health care Institution in Nigeria. The Latitude and Longitude of UCH Ibadan is 7.4065 and 3.9024 respectively. 7.4065 Latitude and 3.9024 Longitude can be mapped to closest address of UCH-Secretariat Road, Ibadan, Nigeria. UCH Ibadan is located in Mokola Hill sub-locality, Ibadan locality, District, Oyo State of Nigeria Country. It has big football pitch for sporting activities, Wards and departments, churches and mosque, and supermarkets for economic activities.

3.3 Target Population

The target population of this study comprised of male and female adult patients (18- 60 years) in university college hospital Ibadan, Oyo state.

3.4 Sampling Techniques

A sample of two hundred (200) respondents was used. Simple random sampling technique was used to select two hundred (200) respondents from five major departments namely: Medicine, Surgery, Orthopeadics, Gyneacology and Emergency department.

3.5 Sample collection

3.5.1 Materials used

- Slide
- Coverslip
- Applicator slide
- Glove
- Normal saline
- Petri dish
- Needle and syringe
- EDTA bottle
- Universal conlaenes
- Lugol's iodine
- Capillary tube
- Macconkey S.shigella agar

3.5.2 Procedure

- A total of 200 blood samples were collected from 200 patients (130 females and 70 males) in university college hospital Ibadan, Oyo state, Southwestern, Nigeria. The samples were collected in sterile containers and transported to the laboratory for processing and to be analyzed.
- The samples were obtained by informed consent of the patients used for this study. Two milliliters of the blood samples were centrifuged at a high speed for 5 min in order to separate the serum from the blood cells.
- The blood culture bottle was incubated at 37oC for 24hrs. Few ml of the cultured blood was taken through neddle and syringe, primary inoculum was made and striking was finally done using Maconkey Agar plate.
- This was carried out repeated for seven days till there was growth on the plate. Gram staining and biochemical tests were carried out as a confirmatory test to confirm the organism isolated procedure for packed cell volume.
- 2/3 of helparinised tube was filled with patients blood, one end of the tube was sealed with plasticine and spured with heamatocrit centrifuge at 50000rpm for 5 mins.
- Results were later read using heamatocrit reader and reported accurately.

3.10 Statistical Analysis

Data collected was analyzed using both descriptive statistical method of analysis for the purpose of this study. A frequency simple percentage was used to present data. Percentage was calculated as number of responses per total number of respondent multiply by 100

Mathematically, $\frac{\text{Number of responses}}{\text{Total number of respondents}} \times \frac{100}{1}$

3.11 Ethical Consideration

An introductory and permission letter from the department was collected by the researcher and taken to the study area to seek permission to conduct the study. Prior to sample collection, the aim and objective of the study was clearly explained to the participants and informed consent was obtained. Also, respondents were assured that the data collected will be used mainly for academic purpose. Confidentiality and anonymity was ensured throughout the execution of the study as participants was not be required to disclose personal information.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

Table 4.1 Demographic Data

VARIABLE	RESPONSES	FREQUENCY	PERCENTAGES
AGE	18-27	44	22%
	28-37	120	60%
	38 and above	36	18%
	TOTAL	**200**	**100%**
EDUCATION	Primary	46	23%
	Secondary	76	38%
	Tertiary	78	39%
	TOTAL	**200**	**100%**
RELIGION	Christianity	116	58%
	Islam	84	42%
	Traditional	00	00%
	TOTAL	**200**	**100%**
TRIBE	Yoruba	166	83%
	Igbo	30	15%
	Hausa	04	02%
	TOTAL	**200**	**100%**
DEPARTMENT	Medicine	55	27.5%
	Surgery	48	24%
	Orthopeadics	45	22.5%
	Gynaecology	22	11%
	Emergency	30	15%
	TOTAL	**200**	**100%**

Analysis from the table above revealed the Socio demographic Data of the respondents. Distribution according to age revealed that 44 (22%) are between 18 and 27years, 120 (60%) are between 28 and 37years while 36 (18%) are 38 and above. On level of education, 46 (23%) had primary education, 76 (38%) had secondary education and 78 (39%) had tertiary education. Distribution according to religion revealed that 116 (58%) are Christians, 84 (42%) are muslims

while none is a traditionalist. Also, 55 (27.5%) are from medicine department, 48 (24%) are from surgery department, 45 (22.5%) are from Orthopeadics department, 22 (11%) are from gynaecology department and 30 (15%) are from emergency department.

Table 4.2. **Frequency distribution of the respondents according to widal sera (salmonella agglutine titres) in relation to sex.**

SEX	NO OF SERA TESTED %	NO OF WIDAL POSITIVE (%)	NO OF WIDAL NEGATIVE (%)
MALE	100 (50)	68 (68)	32 (32)
FEMALE	100 (50)	46 (46)	54 (54)
TOTAL	**200 (100)**	**114 (57).**	**86 (43)**

Analysis from the above table shows that out of the 200 participants of the study, 100 (50%) are males while 100 (50%) are females. 68 % were positive while 32 % were negative among males and 46% were positive while 54% were tested negative among female respondents.

Table 4.3. Frequency distribution of the respondents according to widal sera (salmonella agglutine titres) in relation to its species and PCV level.

SALMONELLAE	NO OF SERA TESTED	NO OF WIDAL POSITIVE (%)	PCV
S. paratyphi A-O	200	56 (28%)	23-30
S. paratyphi B-O	200	48 (24%)	22-27
S. paratyphi C-O	200	63 (31.5%)	23-30
S. typhi O	200	34 (17%)	23-35
S. paratyphi A-H	200	47 (23.5%)	22-31
S. paratyphi B-H	200	31 (15.5%)	23-30
S. paratyphi C-H	200	28 (14%)	23-28
S. typhi H	200	40 (20%)	24-35

Analysis from the table above revealed the specie of Salmonellae present in the tested blood samples. 56 (28%) are tested positive to *S. paratyphi* A-O with PCV between 23-30, 48 (24%) are tested positive to *S. paratyphi* B-O with PCV between 22-27, 63 (31.5%) are tested positive to *S. paratyphi* C-O with PCV between 23-30, 34 (17%) are tested positive to *S. typhi* O with PCV between 23-35, 47 (23.5%) are tested positive to *S. paratyphi* A-H with PCV between 22-31, 31 (15.5%) are tested positive to *S. paratyphi* B-H with PCV between 23-30, 28 (14%) are tested positive to *S. paratyphi* C-H with PCV between 23-28 and 40 (20%) are tested positive to *S. typhi* H with PCV between 24-35.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion of Findings

Analysis from the table above revealed the Socio demographic Data of the respondents. Distribution according to age revealed that 44 (22%) are between 18 and 27years, 120 (60%) are between 28 and 37years while 36 (18%) are 38 and above. On level of education, 46 (23%) had primary education, 76 (38%) had secondary education and 78 (39%) had tertiary education. Distribution according to religion revealed that 116 (58%) are Christians, 84 (42%) are muslims while none is a traditionalist. Also, 55 (27.5%) are from medicine department, 48 (24%) are from surgery department, 45 (22.5%) are from Orthopeadics department, 22 (11%) are from gynaecology department and 30 (15%) are from emergency department. The results of this study revealed that majority of the respondents are mature and educated.

Analysis from table 4.2 shows that out of the 200 participants of the study, 100 (50%) are males while 100 (50%) are females. 68 % were positive while 32 % were negative among males and 46% were positive while 54% were tested negative among female respondents. This shows that samples analyzed for *Salmonella agglutinin* titres were Widal positive among the majority of the participants in this study. This indicates a high prevalence of typhoid fever in the sampled population. However, some of the subjects may not be having the active disease. This is in agreement with the observations of Outi *et al.* (1989) and Adeleke *et al.* (2006) in a similar study on Widal reaction as being more relevance in diagnosing post-infection complications when *S. typhi* may not be isolated. The Widal test reaction involves the use of bacterial suspensions of *S. typhi* and *S. paratyphi* `A' and `B', treated to retain only the `O' and `H' antigens. These antigens are employed to detect corresponding antibodies in the serum of a patient suspected of having typhoid fever. The IgM somatic O antibody appears first and

represents the initial serologic response in acute typhoid fever, while the IgG flagella H antibody usually develops more slowly but persists for longer (Hoffman *et al.*, 1986; Washington and Henry, 1991; Olopoenia *et al.*, 2000). While bacteriological culture remains the gold standard for definitive diagnosis of typhoid fever, lack of its immediate availability during the acute febrile illness may limit its use. In an acute febrile illness in an endemic typhoid region where the clinical picture is ambiguous, a rapid, accurate, specific and sensitive test should be used to differentiate typhoidal from non-typhoidal febrile illnesses. Clinicians usually elect to treat, rather than wait for blood or stool culture results, which may take 3-5 days. While there might be some merit in this approach, particularly in areas where culture facilities are either poor or not available, and where Widal testing is the norm, the use of rapid antigen screening directly from the stool of the suspected patient would be more useful (Olopoenia *et al.*, 2000).

Also in this study, more sera from males were more Widal positive than sera from females. This is probably as a reflection of different eating habits and level of personal hygiene. This is also in agreement with the findings of Adeleke *et al.* (2006). In 380 males, the titre of Salmonella 'O' were higher than those of the 'H' whereas in 460 females, Salmonella 'H' titres were higher than those of 'O'. This differs from what was reported in a similar study by Ibekwe *et al.* (2008) where 82 apparently normal males had higher titre of Salmonella 'H' and 118 apparently normal females had higher Salmonella 'O' titres (Ibekwe *et al.*, 2008).

Furthermore, analysis from table 4.3 revealed the specie of Salmonellae present in the tested blood samples. 56 (28%) are tested positive to *S. paratyphi* A-O with PCV between 23-30, 48 (24%) are tested positive to *S. paratyphi* B-O with PCV between 22-27, 63 (31.5%) are tested positive to *S. paratyphi* C-O with PCV between 23-30, 34 (17%) are tested positive to *S. typhi* O

with PCV between 23-35, 47 (23.5%) are tested positive to *S. paratyphi* A-H with PCV between 22-31, 31 (15.5%) are tested positive to *S. paratyphi* B-H with PCV between 23-30, 28 (14%) are tested positive to *S. paratyphi* C-H with PCV between 23-28 and 40 (20%) are tested positive to *S. typhi* H with PCV between 24-35. The value of Widal test depends upon the standardization and maintenance of the antigens to produce consistent results, and it has become evident from work done in recent years on standardization of the Widal test and interpretation of the results that both the O and H antigens are necessary for proper serologic analysis of the suspected serum. However, according to Welch in 1936 (reviewed in Olopoenia *et al.*, 2000), no Widal test, regardless of the composition and standardization of the antigens used, is infallible, and thus it is unlikely that any will be developed that will lower the validity of the isolation of the aetiologic agent. Sansone *et al.* (1972) published a case report where the Widal reaction to typhoid O antigen on admission for an unexposed patient was 1:320, with an increase in titre to 1:20 480 by the fourth day. In an individual with no prior exposure to *S. typhi* infection (either lack of active infection or absence of passive immunization), a higher than 1:50 or 1:100 titre on an initial single test, usually correlates fairly well with exposure to typhoid fever (Olopoenia *et al.*, 2000). However, even these single high-value titres in an endemic area where repeated exposures to *S. typhi* may have occurred, do not have any clinical relevance in the absence of a positive isolate of the causative organism or its antigen. A second sample collection will proof useful. But, in a situation where second sample collection is not feasible, knowledge of the agglutinin levels in the sera of normal subjects from the patients' community can form the baseline on which a diagnosis can be made (Opera and Nweke, 1991; Ibekwe *et al.*, 2008).

Also, a negative agglutination test may be for one of several reasons which include: 1) absence of infection by *S. typhi*, 2) the carrier state, 3) an inadequate inoculum of bacterial antigen in the

host to induce antibody production, 4) technical difficulty or errors in the performance of the test, 5) previous antibiotic treatment and 6) variability in the preparation of commercial antigens. A negative Widal test result does not therefore necessarily rule out the absence of infection. Such results are best kept as a reference for subsequent comparative analysis (Olopoenia *et al.*, 2000). A positive agglutination tests (on two successive occasions) on the other hand, may also be open to several different interpretations. 1) the patient being tested has typhoid fever, 2) previous immunization with *Salmonella antigen*, 3) cross-reaction with non-typhoidal Salmonella, 4) variability and poorly standardised commercial antigen preparation, 5) infection with malaria or other enterobacteriaceae, 6) other diseases such as dengue (Olopoenia *et al.*, 2000). This could lead to confusion in the serological diagnosis of typhoid fever. Therefore, serological findings have to be interpreted with a lot of caution particularly in country like Nigeria where there are yet to be laid down standard baseline titres (Ibekwe *et al.*, 2008).

Moreso, in endemic typhoid regions, a single testing of a serum specimen for Widal agglutinin cannot provide a reliable diagnosis due to: repeated exposure to small inocula of *S. typhi* or to other *Salmonella* spp. that contain type 9 or 12 antigens, previous typhoid fever immunization and other infectious agents such as malaria (Olopoenia *et al.*, 2000). Although a number of reports from some developing countries have suggested that a single Widal test is sufficient to make the diagnosis of typhoid fever (Mohammed *et al.*, 1992; Rasaily *et al.*, 1993; Choo *et al.*, 1993), others have disputed the usefulness of such a single test result (Hoffman *et al.*, 1986; Aquino *et al.*, 1991). In some developing countries where the use of a single Widal test appears to be the norm, there has been an increase in the rate of falsepositive results (Olopoenia *et al.*, 2000).

Typhoid and paratyphoid fevers are common in less industrialized countries, principally owing to the problem of unsafe drinking-water, inadequate sewage disposal and flooding. Public health interventions to prevent typhoid and paratyphoid include: 1) health education about personal hygiene, especially regarding hand-washing after toilet use and before food preparation; provision of a safe water supply; 2) proper sanitation systems; 3) excluding disease carriers from food handling. Control measures to combat typhoid include health education and antibiotic treatment. A vaccine is available, although it is not routinely recommended except for those who will have prolonged exposure to potentially contaminated food and water in high-risk areas. The vaccine does not provide full protection from infection (WHO, 2008).

The review of Olopoenia *et al.* (2000) and Adeleke *et al.* (2006) suggesting Widal agglutination test as being bedeviled with controversies in term of quality of *Salmonella antigens* and interpretation of results is also pertinent. It should be stressed that a single Widal agglutination test has no diagnostic significance.

According to Hoffman *et al.* (1986), the results of a single Widal test, tube dilution, micro-agglutination or slide agglutination are virtually un-interpretable unless the sensitivity and specificity of the test for the specific laboratory and patient population are known, as well as predictive values. Even in the extreme case of a high titer in a single Widal agglutination test, the causative organism may often be due to other species of *Salmonella*, rather than *S. typhi* (Olopoenia *et al.*, 2000). Thus, for a more definite diagnosis of typhoid fever, serologic test and blood culture as well as stool culture from every patient are quite relevant. Therefore, efforts must be made however, to confirm the diagnosis by paired sera investigation more than in presently the case.

5.2. Conclusion

In conclusion, it is clear that *Salmonella typhi* low packed cell volume is common among patients. Obviously therefore, the prevalence of typhoid fever and the increasing menace of Multi-Drug Resistance (MDR) of its causative agent are seriously constituting a menace in poor developing tropical countries. The resultant effect on health status would affect productivity, intellectual development and other aspects of life. There is therefore an urgent need, for measures to curtail the spread of the disease. Serologic studies are helpful in typhoid fever cases in endemic regions only if patients have four-fold or greater increases in O or H agglutinin titres in serum specimens obtained 2-3 weeks apart.

5.3 Recommendations

Based on the findings of this study, the following recommendations were made:

- There should be close communication between the physician requesting the test and the laboratory, since modifications of technique in individual laboratories may affect the Widal titres and some patients with bacteriologically confirmed typhoid fever may fail to develop the usual rise of antibody titres.
- The results of the tests should be reported as either 'no agglutination' or, if agglutination is present, in titres (1:20, 1:40 or 1:80) rather than in descriptive (negative or positive) terms, as the latter may be misleading and contribute to the false interpretation of the test result by the physician.
- The laboratory personnel should perform and report the test result to the requesting physician who will use the data to help make the proper diagnosis and prescribes the antibiotics.

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