

Nigerian  
**Biomedical Science Journal**

ISSN 0794-1056

—Nigeria Foremost Journal of Biomedical Practice.—

Jan. - Mar. 2024

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# An Evaluation of The Bacterial Quality of Cow, Chicken and Goat Meat Sold in Owerri Municipal Area, Imo State, Nigeria.

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## ABSTRACT

The poor hygienic state of our abattoirs and markets where meats are sold have posed a major threat to public health. The bacteriological quality of meats sold in Owerri municipal area of Imo State, Nigeria was investigated with the aim of determining the bacterial loads of meat sold in these areas. Samples numbering 110 were collected from 11 meat samples types sourced from cow, goat, and chicken. The eleven (11) meat sample types were fresh cow muscles (FCM), fresh cow intestine (FCI), fresh cow liver (FCL), and fresh cow towel (FCT). Fresh goat muscles (FGM), fresh chicken muscle (FCM), fresh goat intestine (FGI), fresh chicken intestine (FCI), Fresh goat liver (FGL), fresh chicken liver (FCL), fresh goat towel (FGT) etc. The samples were collected with sterile containers and swab sticks. The bacteriological analysis were done using pour plate method on Nutrient Agar, MacConkey Agar and Salmonella shigella Agar. The colonies were counted, characterized and identified using standard microbiological and biochemical techniques. The organisms isolated were *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, *Klebsiella* specie and *Coliform* specie. Fresh chicken intestine has the highest total mean bacteria count of  $1.1 \times 10^7$  (cfu)/ml, followed by fresh cow towel  $7.0 \times 10^6$  (cfu)/ml and lastly fresh chicken liver  $8.2 \times 10^5$  (cfu)/ml. The presence of all these organisms can pose a major threat to public health. This calls for regular inspection of animals, abattoir environments and regular health check of abattoirs workers and butchers.

**Key words; Bacterial; Salmonella; E. Coli. Klebsiella**

## INTRODUCTION

Meat contains high quality of protein, fat, carbohydrate, vitamins and minerals which are delicious and easily digestible food (Lee *et al.*, 2020). All these nutritional requirements can be met easily if reasonable amount of meat is included in a diet. Meat is an animal flesh that is eaten as food and is an excellent source of protein in human diet (Lawrie and Ledward, 2006). Its chemical composition is ideal for the growth of a wide range of spoilage and pathogenic bacteria. Chemical composition includes 72-75% of water, 21% Nitrogen compounds (19% proteins and 1.6% non-protein Nitrogen), 25-50% lipids, 1% of non-nitrogenous compounds (vitamins) and carbohydrate (very small amount of glycogen). Contaminated raw meat is one of the main sources of food-borne illnesses. According to Paul *et al.*, (2014), food borne diseases result from ingestion of bacteria, toxins present in the meat. The intensity of symptoms varies with the amount of contaminated meat ingested and the susceptibility of the individual to the toxin. These can results in economic and health losses (Gupta, 2017, Sharif *et al.*, 2018).

Meat is gotten from animal like goat, cattle, chicken, etc, and they have essential parts that are used as meat e.g beef (red meat), intestines (small and large intestine), and the skin which is also called node. Muscles of healthy animals do not contain micro-organisms, meat tissue get contaminated during the various stage of slaughter and transportation. The health status of animals prior to slaughtering and prevailing circumstances in the slaughter house contributes to the quality of meat from such animals (Whyte *et al.*, 2004). In Nigeria particularly, Owerri in Imo state, slaughtering of animals usually takes place under very unhygienic conditions. This coupled with the high ambient

temperature, high humidity, shortage of portable water and poor handling practices, exposes meat to microbial contamination (Whyte *et al.*, 2004).

Other primary sources of microbial contaminations are the equipments and physical facilities (stock, knives, containers, retail tables) used in each operation before the final is eaten (Jay *et al.*, 2005, Bakhtyari *et al.*, 2016, Rouger *et al.*, 2017).



A selection of uncooked red meat and poultry, including beef and chicken. Source; By Unknown photographer - <https://visualsonline.cancer.gov/details>.

Spoilage is caused by the practically unavoidable infection and subsequent decomposition of meat and by bacteria and fungi, which are borne by the animal itself, and the people handling the meat and their equipment. A great diversity of microbes inhabits fresh meat generally, but different types may become dominant depending on PH (Jay *et al.*, 2005).

composition, textures, storage temperature and transportation method involves (Ercoloni *et al.*, 2006). Raw meat may harbor many important pathogenic microbes such *Salmonella* species, *Campylobacter Jejuni*, *Yersinia*, *Escherichia coli*, *Staphylococcus aureus* and to extent, *Listeria monocytogenes*, making the meat a risk for human

health of which without proper handling and control of these pathogen, food borne illness may occur. (Manninen, *et al.*, 2002). Therefore, this work is aimed at evaluating the bacterial quantity of cow, goat and chicken meat sold in Owerri municipality, Imo state, Nigeria.

## Materials and Methods

### Study setting

The study was conducted in Owerri Municipal Council area of Imo State, Nigeria. Owerri is the capital of Imo State which is located in South Eastern Nigeria, lies between latitude 5°51 North, Longitude 6°35 and 7°28 east and comprises 27 local government areas with a total land mass of 5,289.49km<sup>2</sup> and a total population of 3,928,634 (Imo State Statistical year book, 2011). Imo State is made up of both civil servants and business men and their main occupation is farming and trading. The population is made up of indigenes, tenants, business men and civil servants.

### Sample collection, preparation and analysis

Fresh beef, chicken and goat meat was purchased from different butchers, slaughter houses, open shops, markets within Owerri Municipality which includes Ekeonuwa market, Relief market, New market, etc. Samples were promptly transported to Medical Microbiology Laboratory, Imo State University, with insulated ice container for bacteriological analysis.

The method described by (Jay *et al.*, 2005) was adopted in the preparation of the meat samples. One (1) grams of each of the solid samples was weighed and aseptically taken into a sterilized jar containing 9mls sterile distilled water to produce a stock solution or homogenized solution through blending at 300rpm for 5 minutes. An aliquot of homogenized solution was transferred for a test containing 9ml of sterile water to make a tenfold serial dilution and was shaken vigorously. Sterile dilutions up to 10<sup>-5</sup> was prepared for microbiological analysis.

### Laboratory analysis

The bacterial quality of the meat was assessed on the basis of Total Viable Bacterial Count (TVBC), Total *Coliform* Count (TCC), Total *Salmonella* and *Shigella* (TSSC), and Total *Staphylococcus* Count (TSC) using Nutrient Agar, MaConkey Agar and *Salmonella-Shigella* agar respectively. 1ml of homogenized solution was put into a sterile petridish aseptically followed by addition of the appropriate medium (19m/s), allowed to solidified at room temperature and incubated at 37°C 24hrs. The bacterial isolates from the plates were further identified by gram staining other biochemical tests such as catalase, oxidase, indole, etc. as described by Cheesebrough, (2002) and Ochei and Kolhatkar, (2000).

### Statistical analysis

The data obtained from this study were presented and analyzed using charts and frequency distribution.

### Results

A total of 110 fresh meats samples from 11 meat sample types sourced from cow, goat and chicken. The Eleven (11) sample type namely fresh cow muscles (FCM), fresh cow intestine (FCI), fresh cow liver (FCL), fresh cow towel (FCT), fresh chicken muscles (FCHM), fresh chicken intestine (FCHI), fresh chicken liver (FCHL), fresh goat muscles (FGM), fresh goat intestine (FGI), fresh goat liver (FGL), and fresh goat towel (FGT) were collected from different meat vendors at abattoir and major markets in Owerri Municipal, Imo State, Nigeria and analyzed bacteriological,

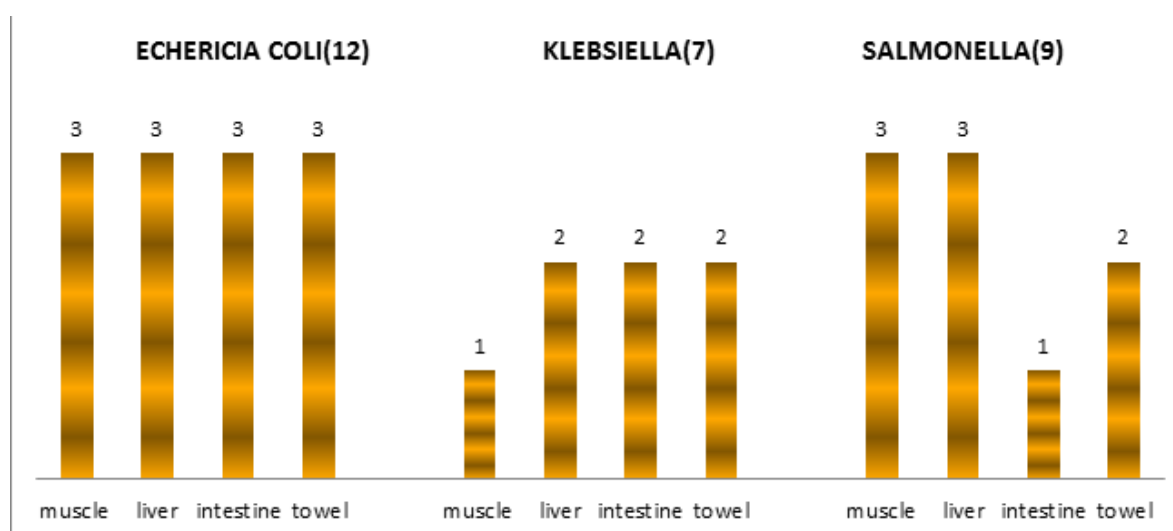


Figure 1: occurrence of pathogenic bacteria species in goat meats sold in owerri

Figure 1 reveals that *Escherichia coli* has the highest occurrence of (12) followed by *Salmonella* (9) and finally *Klebsiella* (7).

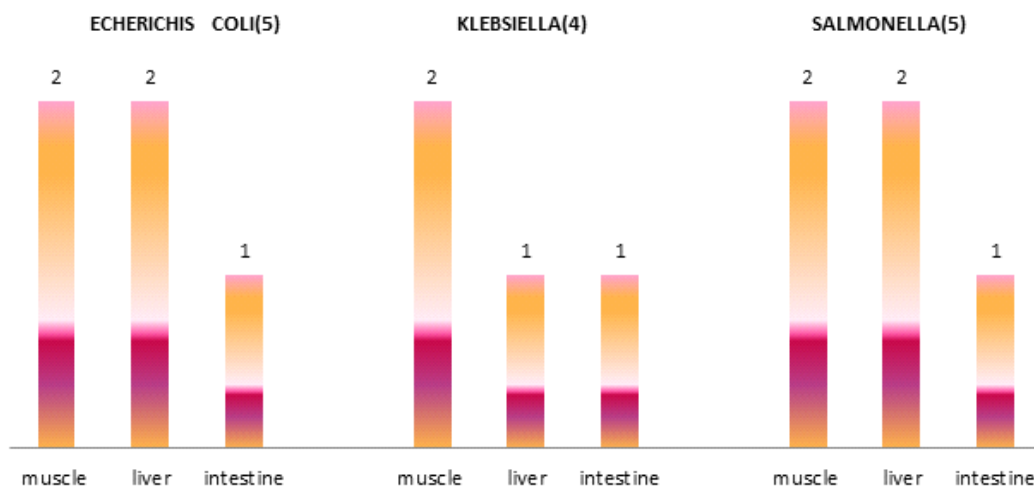


Figure 2: Occurrence of pathogenic bacteria species in chicken meat sold in Owerri

Figure 2 shows that *Salmonella spp* and *E. coli* have the highest occurrence of (5) each followed by *klebsiella spp* (4). This is because *salmonella species* are mostly found in poultry.

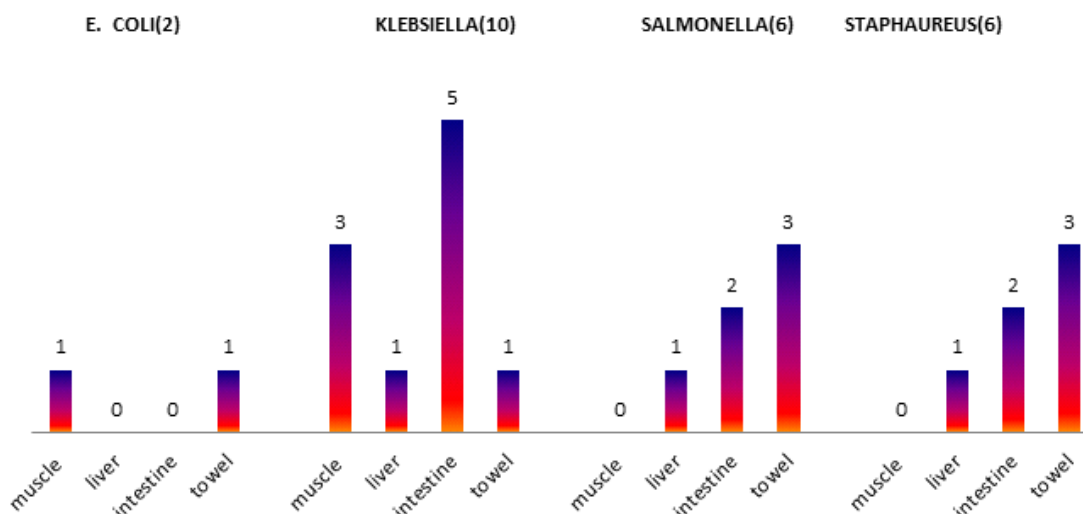


Figure 3: Occurrence of pathogenic bacteria species in Cow meat sold in Owerri.

Figure 3 show that *Klesiella spp* has the highest occurrence of (10) followed by *Salmonella* and *staphylococcus aureus* (6) each, finally *E. coli* (2).

In Table 1: Shows the variation of microbial loads of fresh meat samples. Fresh chicken intestine has the highest total mean bacteria count of  $1.1 \times 10^7$  (cfu)/ml followed by fresh cow towel  $7.0 \times 10^6$  (cfu)/ml and lastly fresh chicken liver  $8.2 \times 10^5$  (cfu)/ml. This may be as a result, chicken are been raised in a large scale farm and come in contact with many other chickens which increase the spread of bacteria.

According to Bacterial Analytical Manual (BAM) and American Society for testing materials says that 25 – 250(cfu)/ml plate and 100,000 colonies /ml are significant for infection respectively. Therefore, the result from table below is significant for infection.



TABLE 1: SHOWS THE VARIATION OF MICROBIAL LOADS OF FRESH MEAT SAMPLES.

| MEAT SAMPLES | TMVBC (cfu/m)       | TMCC                | TMSSC               | TMEC(cfu/m)         | TMSC                | TMKC                |
|--------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| FCM          | 1.1×10 <sup>4</sup> | ND                  | 3.6×10 <sup>4</sup> | 2.0×10 <sup>4</sup> | ND                  | ND                  |
| FCL          | 2.0×10 <sup>4</sup> | ND                  | 4.0×10 <sup>3</sup> | 1.5×10 <sup>4</sup> | 5.0×10 <sup>3</sup> | ND                  |
| FCI          | 6.0×10 <sup>4</sup> | ND                  | 4.3×10 <sup>3</sup> | 9.3×10 <sup>3</sup> | 1.0×10 <sup>6</sup> | ND                  |
| FCT          | 7.0×10 <sup>6</sup> | ND                  | 7.5×10 <sup>3</sup> | 6.0×10 <sup>6</sup> | 8.0×10 <sup>5</sup> | 2.5×10 <sup>4</sup> |
| FGM          | 3.8×10 <sup>5</sup> | ND                  | 1.1×10 <sup>5</sup> | 5.9×10 <sup>5</sup> | ND                  | 3.1×10 <sup>5</sup> |
| FGL          | 5.0×10 <sup>5</sup> | ND                  | 3.5×10 <sup>3</sup> | 3.0×10 <sup>5</sup> | ND                  | ND                  |
| FGI          | 4.1×10 <sup>5</sup> | ND                  | 5.0×10 <sup>3</sup> | 4.1×10 <sup>5</sup> | ND                  | 5.9×10 <sup>4</sup> |
| FGT          | 9.0×10 <sup>4</sup> | ND                  | 4.9×10 <sup>5</sup> | 6.5×10 <sup>4</sup> | ND                  | 1.3×10 <sup>5</sup> |
| FCHM         | 1.6×10 <sup>6</sup> | 3.5×10 <sup>5</sup> | 3.4×10 <sup>5</sup> | 41×10 <sup>5</sup>  | ND                  | 4.4×10 <sup>5</sup> |
| FCHL         | 8.2×10 <sup>5</sup> | 4.8×10 <sup>5</sup> | 9.1×10 <sup>3</sup> | 3.3×10 <sup>5</sup> | ND                  | 6.0×10 <sup>5</sup> |
| FCHI         | 1.1×10 <sup>7</sup> | 1.8×10 <sup>5</sup> | 4.5×10 <sup>5</sup> | 2.0×10 <sup>5</sup> | ND                  | 1.0×10 <sup>6</sup> |

**KEY:**

TMVBC: Total Mean Viable Count; TMCC: Total Mean Coliform Count; TMSSC: Total Mean Salmonella shigella count; TMEC: Total Mean Escherichia coli count; TMSC: Total Mean Staphylococcus count.; FCM: Fresh Cow Muscles, FCL: fresh cow liver,; FCI: Fresh Cow Intestine, FCT: fresh cow towel; FGM: Fresh Goat Muscles, FGL: fresh goat liver; FGI: Fresh Goat Intestine, FCHM: fresh chicken muscles; FCHL: fresh chicken liver, FCHI: fresh chicken intestine ; Cfu/ml: Colony Forming Unit per Milliliter.

Table 4.3 shows the overall bacteria isolated and their percentage distribution. *Eschericia coli* has the highest frequency and percentage distribution 36 (43%) followed by *Salmonella* species 24(28%), *Klesbsiella* 13 (15%), *Staphylococcus aureus* 6 (7%) and lastly *Coliform species* 5 (6%)

*E. coli* is an indicator of fecal contamination as well as undercooked meat and this is in line with Ozelen, 2005. This can lead to bloody diarrhea, vomiting and bloody dysentery.

TABLE 4.3: SHOWS THE OVERAL BACTERIA ISOLATED ON DIFFERENT MEAT SAMPLES AND THEIR PERCENTAGE DISTRIBUTION

| BACTERIA ISOLATED            | GOAT | COW | CHICKE N | FREQUENC Y | DISTRIBUTION % |
|------------------------------|------|-----|----------|------------|----------------|
| No. of samples               | 40   | 40  | 30       | 110        |                |
| <i>Eschericia coli</i>       | 12   | 19  | 15       | 36         | 43%            |
| <i>Klebsiella spp</i>        | 7    | 2   | 4        | 13         | 15%            |
| <i>Salmonella spp</i>        | 9    | 5   | 10       | 24         | 28%            |
| <i>Coliform spp</i>          | --   | --- | 5        | 5          | 6%             |
| <i>Staphylococcus aureus</i> | --   | 7   | ---      | 6          | 8%             |
|                              |      |     |          | 84         | 100%           |

### Discussion

In this study, a total of five (5) species of bacteria were seen in goat, chicken and cow. These species are identified as *Eschericia coli*, *Klebsiella spp. coliform*, *Staphylococcus aureus* and *Salmonella spp.* The frequency and percentage distribution of bacteria isolates from fresh meat marketed in Owerri municipal showed that cow muscle parts has the highest total bacteria species 13 (35%) while chicken and goat have the same frequency and percentage distribution of 12 (32%). Among these bacterial species, *Eschericia coli* has the highest percentage distribution of 43% and highest total mean viable count in cow towel, chicken intestine, goat towel and cow towel ( $7.0 \times 10^6$ (cfu/ml), ( $8.2 \times 10^5$ (cfu/ml), ( $9.0 \times 10^4$  (cfu/ml) and  $6.4 \times 10^4$  (cfu/ml) respectively. *Eschericia coli* is assumed to be an indicator of faecal contamination. This is in agreement with Ozlem, (2005) and Haggard *et al.*, 2004) which says that *Eschericia coli* contamination is as a result of faecal contamination of the samples. This may be attributed to improper sanitary condition during processing of meat from water supply, unsterilized utensils and contaminants from flies.

*Salmonella* species has the second highest percentage distribution of 28% and highest total mean viable count in fresh goat towel  $4.9 \times 10^5$ (cfu/ml, chicken intestine  $4.5 \times 10^6$ (cfu/ml and chicken muscle  $3.4 \times 10^5$ (cfu/ml etc. This result is in contrast with the work of (Mgbemena *et al.*, 2015) that reported that no *Salmonella* and *Shigella* were seen in the samples examined. *Salmonella species* such as *Salmonella typhi* is a bacterium that causes typhoid fever, and acute life threatening ulcer. The disease is a cause of concern and major public health problem in underdeveloped countries (Asia and Africa) especially in Nigeria due to poor sanitary conditions and lack of portable water. *Coliforms* was seen in fresh chicken muscles ( $1.6 \times 10^6$ (cfu/ml), intestine ( $1.1 \times 10^7$ (cfu/ml) and liver ( $8.2 \times 10^5$ (cfu/ml). This may be as a result of reused dirty water in washing meat as well as dirty environment where the chicken is slaughtered.

This work has revealed that microbial qualities of meat sold in Owerri municipal market are grossly contaminated by gram positive and gram negative but mostly gram negative bacteria. The organisms isolated are in line with the work of Shrestha *et al.*, (2017) and Baah *et al.*, (2022), who reported that gram negative account for approximately 69% of the cases of food borne bacterial diseases. The presence of all these organisms on meat is indicative of public health hazard and gives a signal of possible occurrence of food borne intoxication and infection. This also implies that these meats are viable source of various diseases. The presence of *Staphylococcus aureus* in this study is as a result of cross contamination from many butchers before it gets to the final retailers. *Staphylococcus* being a flora of the body indicates contamination from the handlers. The organism can pass onto meat during slaughtering, butchering and evisceration (Tegegne, *et al.*, 2023, Naas *et al.*, 2019). It is the major cause of food poisoning (Şanlıbaba, 2022). The poisoning is caused by ingestion of an enterotoxin produced which is characterized by diarrhea and vomiting (Rusnak *et al.*, 2004, Liu, *et al.*, 2022, Yuan *et al.*, 2023)

### Conclusion

The findings in this study has shown a high bacterial load in meat sold in Owerri Municipal area in which *Eschericia coli* have the highest percentage distribution.

*Eschericia coli*, *Staphylococcus aureus* and others have been the aetiological agents of food-borne diseases. According to WHO, these organisms are “priority organisms”. They are involved in multidrug resistant bacteria which increases mortality rate. These pose great challenge to public health as it may serve as a source of food poisoning and death. Therefore, consumers should apply proper cooking methods to reduce microbial load.

### RECOMMENDATIONS

. In this study, we recommended that public health inspectors and veterinary doctors should be recruited for regular inspection of animals, meat abattoir environments and regular health check of butchers and abattoirs workers.

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# Effects of Lowenstein- Jensen Medium on *Mycobacterium Tuberculosis* Growth

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## ABSTRACT

**M**ycobacterium tuberculosis (MTB) growth rate on Lowenstein- Jensen (L-J) medium takes 21 to 60 days. This study modified the L-J medium to achieve a faster growth rate. Both conventional and modified L-J powder containing potato flour (L-J modification 1) were used at the preliminary improvement stage. It was observed that the higher the concentration of the egg, the faster the smears from the L-J slants showed acid fast bacilli (AFB) positive results. Modified L-J slants containing 70% and 75% egg yielded MTB growth in 5 -8 days. The modified L-J 2 medium contained 70% egg and 0.5 g/L of sodium citrate, without the potato flour present in the conventional L-J medium. Because MTB growth occurred in 5 days in the modified L-J 2 medium, it would be possible for both MTB isolation and antibiotic sensitivity test to be completed in less than 14 days. Thus the modified L-J medium 2 is recommended for quicker isolation and confirmation of the MTB cases. Conventional L-J contained low quantity of egg than the modified L-J 1 and 2. It also contained higher potatoe flour than modified L-J. Modified L-J contained no potatoe flour but contained 70ml of fresh egg.

**Keywords:** Tuberculosis, TB<sub>Ag</sub>MPT64, *Mycobacterium*, Acid Fast bacilli, Lowenstein-Jensen Medium.

## INTRODUCTION

**B**ackground The history of tuberculosis (TB) infection dates along with the human history, and TB probably have been existing over 70,000 years ago but remained sporadic to date (Luise, 2012). Its infection becomes complicated when co-infected with Human Immunodeficiency Virus (HIV) (Singh, 2019).

Concealment of *Mycobacterium tuberculosis* (MTB) infection, drunkenness or alcoholic consumption, carefree attitudes towards its means of transmission, and superstition have all made the spread of TB rapid (Kaufmann *et al.*, 2013). TB-HIV coinfection, weakened potency of Bacillus Calmette Guerin (BCG) vaccine and massive migration from rural to urban cities and across continental boundaries led to emergency of multidrug resistant TB (MDR-TB) Kaufmann (2013). Transmission of TB is via coughing, sneezing, shouting, crying, laughing, smoking cigarette or drinking from the same bowl with the TB infected patient. Poor or non-notification of TB infection with inadequate awareness also aids spread of TB. Some of them may be drug resistant, multi-drug resistant or extensive drug resistant tuberculosis (Sanchez *et al.*, 2012). Tuberculosis should be treated promptly with respect and dignity the patient deserves (Philipp *et al.* 2011).

The greatest challenge to TB infection is isolation due to its slowness in growing in L-J medium that takes 21 to 60 days, and has identification problem also (Padmawali *et al.*, 2016; Muril *et al.*, 2022 and Sagar, 2022). The disease is spreading very fast beyond the medical control measures against its infection (Adegboyaga *et al.* 2014).

To overcome its isolation challenge, a modification of TB growth medium will be considered in this research to improve the growth rate and identification process.. The aim of this research is therefore to improve L-J medium to facilitate faster growth of MTB, identify the *Mycobacterium tuberculosis* complex (MTBC) and determine the rifampicin resistant MTB isolates.

## MATERIALS AND METHODS

### Sample Collection

Five hundred sputum samples were collected from 265 (53%) males and 235 (47%) females who were screened for *Mycobacterium tuberculosis* (MTB). The Ziel-Neelsen's (ZN) staining method was used to screen for MTB from the sputum samples in this study.

### Culturing of the Sputum Samples

Conventional L-J medium (37.24g) was weighed, dissolved in 600mL of distilled water containing 12 mL of glycerol. 70ml of fresh whole egg was aseptically washed, carefully cracked into 1-litre measuring cylinder, mixed carefully and blended aseptically in a sterile blender. The L-J medium was autoclaved at 121°C, 15psi for 15 minutes. When it cooled to about 50°C, the blended 70ml of fresh whole egg was added and mixed by swirling. 10ml each were then dispensed into sterile McCartney bottles, slanted, and inspissated at 80°C for one hour daily for 3 consecutive days.

### Purification and Liquefaction of the Sputum Samples

Freshly prepared 4% sodium hydroxide (NaOH) was prepared with sterile distilled water (SDW). Equal volume of sputum sample was mixed with NaOH, incubated at 37°C for 20 minutes. The sputum-NaOH solution was mixed by turning the container upside down three times after every 5 minutes for 20 minutes. The samples were spun at 3000 rpm for 20 minutes in a Bio-Safety cabinet. The supernatant was carefully discarded into a solution of 5% Lysol or 10% freshly prepared sodium hypochlorite while the deposit was suspended in sterile distilled water (SDW).

### Modification of L-J Medium

Modified L-J 1 powder included potato flour while modified L-J 2 excluded potato flour and included sodium citrate. The modified L-J medium that was formulated contained asparagine 7.5g/L; dipotassium phosphate ( $\text{KH}_2\text{PO}_4$ ) 5.0g/L; magnesium citrate 1.2g/L; magnesium sulphate 0.5g/L; malachite green 0.3 g/L, sodium citrate 0.4g/L as well as 7.4 mL/L of glycerol, 292.6 mL/L of SDW, 700mL/L of fresh, 4-day old whole egg and 690mg/L of amoxicillin, 690 mg/L of difflucan, 690mg/L of gentamycin, 690 mg/L of azithromycin, 690 mg/L of nalidixic acid and 690 mg/L of ciprofloxacin. During the preparation, the modified L-J medium was dissolved in SDW, glycerol was added into the solution and they were sterilized at 121 °C, 15 psi pressure for 15 minutes. When it cooled to 50 °C, the fresh 4-day old blended whole egg was added and mixed by gentle swirling without forming air bubbles. With sterile McCartney bottles, slants were made and inspissated at 80 °C for 1 hour for 3 consecutive days.

### Inoculation of the Slants

With a sterile micropipette, 10µL of purified, extracted Mycobacteria was inoculated unto the slants. Four hundred and sixty-three purified sputum samples were inoculated on the modified L-J and 50 of the same were inoculated into the conventional L-J to serve as controls. They were incubated at 37°C aerobically and monitored every 24 hours

### Identification of the Isolated MTB

With sterile wireloop a colony or two was picked, emulsified on a clean microscope slide, stained with ZN method and examined with ×100 objectives and immersion oil.

TBAgMPT<sub>64</sub> tests were carried out on grown MTB to confirm MTB complex while GeneXpert was used to confirm rifampicin resistant MTB in sputum samples. Drug sensitivity tests of the MTB isolates was done with modified

L-J medium.

### Procedures:

TBAgMPT<sub>64</sub> with 100ml reagent buffer pipetted into a test tube, one or two colonies of MTB was emulsified and 100µl of the mycobacterial suspension siphoned and pipetted into the TBAgMPT<sub>64</sub> testing capsule and left for 20min at 25°C - 30°C.

### GeneXpert:

10ml of Xpert buffer was added into the cup containing the sputum and lightly colored the cup. The cup was vigorously shaken with back and forth movement for 10 times and left at 25°C - 30°C for 20min. Additional 10ml of the buffer was added and shaken repeatedly where the sputum was not completely liquefied. The liquefied sputum was siphoned to 2ml and placed into the GeneXpert cartridge. The cartridge was inserted with the GeneXpert machine. The machine was switched on to run for 1hr, 50min. At completion, the machine stopped automatically and the results were recorded.

### RESULTS

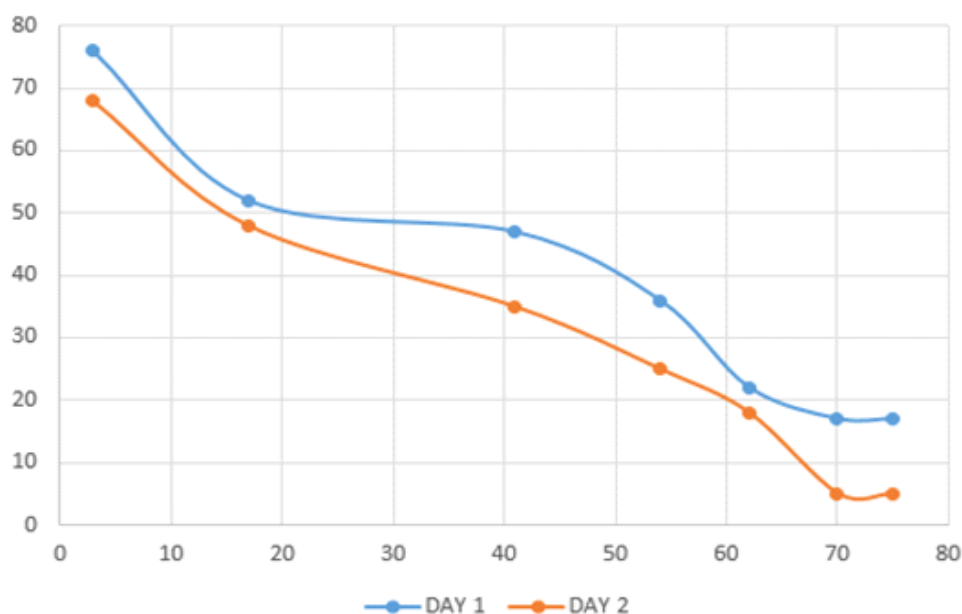
Modified L-J 1 was the preliminary medium formed with potato flour. MTB grew on both improved L-J medium but grew faster on the improved L-J 2 slant without potato flour

### Key:

L-J = Lowenstein-Jensen

Five hundred sputum samples were collected. The growth time (days) plotted against the concentration of egg (in percentage) is represented in Figure 1.

Growth time (Days)



### Keys

—●— Modified L-J medium 1    —●— Modified L-J medium 2



### Fig 1: Change in Mycobacterial growth period (days) with egg concentration (%)

The growth of Mycobacteria on 70% egg modified L-J 1 medium containing potato flour appeared on day 17 but that of the modified L-J 2 medium without potato flour appeared on the 5<sup>th</sup> day. From the growth curve (Fig 1), the lowest concentration of egg (3%) grew MTB in 76 days on modified L-J 1 medium while the growth of MTB at the same concentration in modified L-J 2 medium grew MTB on the 68<sup>th</sup> day.

### Identification of Mycobacterial Isolates

Table 1 indicates the results obtained from 500 sputum samples. From the ZN results, all the 463 MTB isolates were ZN positive, TBAGMPT<sub>64</sub> test detected 443 MTBC positive and 15 negative while GeneXpert result indicated 427 MTB with 423 MTB/Rifampicin sensitive (RS), 2 MTB/Rifampicin resistant (RR) and 2 MTB/Rifampicin indeterminate (RI). 37 patients samples did not show ZN positive, no growth of MTB on the L-J media and no positive with GeneXpert or TBAGMPT<sub>64</sub> tests. Thus, they had no TB infection.

**Table 2: MTB isolates from 500 samples on L-J, L-J 1, L-J 2, ZN stain, MPT<sub>64</sub> and GeneXpert**

| Isolates |             | Ziel Neelsen |          | TBAGMPT <sub>64</sub> |         | GeneXpert   |           |
|----------|-------------|--------------|----------|-----------------------|---------|-------------|-----------|
|          |             | (+)          | (-)      | (+)                   | (-)     | (+)         | (-)       |
| MTB      | 463 (92.6%) |              |          |                       |         |             |           |
| No MTB   | 37 (7.4%)   | 0 (0%)       | 0 %      | 0                     | 0       | 0%          | 0%        |
| L-J      | 130 (26%)   | 130 (26%)    | 0 %      | 120 (24%)             | 10 (2%) | 112 (22.4%) | 18 (3.6%) |
| L-J1     | 133(26.6%)  | 133 (26.6%)  | 0 %      | 128 (25.6%)           | 5 (1%)  | 125 (25%)   | 8 (1.6%)  |
| L-J2     | 200 (40%)   | 200 (40 %)   | 0 %      | 195 (39%)             | 5 (1%)  | 190 (38%)   | 10 (2%)   |
| Total    | 500 (100%)  | 463 (92.6%)  | 37(7.4%) | 443 (88.6%)           | 20 (4%) | 427 (85.8%) | 36 (7.2%) |

### Keys

No Exam = Total no of Samples worked on. MTB isolate = Total no of MTB isolated from the L-J culture

L-J = MTB isolated from Conventional L-J Medium  
(+) = Positive

L-J 1 = MTB isolated from mod. L-J 1 (-)

= Negative

L-J 2 = MTB isolated from mod. L-J (2)

L-J =

Lowenstein–Jensen medium

No MTB = Samples that grew no MTB, nor GeneXpert positive

Z N = Ziel - Neelsen  
MPT<sub>64</sub> = TBAGMPT<sub>64</sub>

**Table 3: Drug Sensitivity Patterns of First Line anti-TB drugs**

| Drug         | No tested         | Sensitive          | Resistant       |
|--------------|-------------------|--------------------|-----------------|
| Rifampicin   | 93 (20%)          | 93 (20%)           | -               |
| Ethambutol   | 92 (19.9%)        | 90 (19.4%)         | 2 (0.4%)        |
| Isoniazid    | 93 (20%)          | 91 (19.7%)         | 1 (0.2%)        |
| Streptomycin | 92 (19.9%)        | 92 (19.9 %)        | -               |
| Pyrazinamide | 93 (20%)          | 93 (20%)           | 0               |
| <b>Total</b> | <b>463 (100%)</b> | <b>460 (99.4%)</b> | <b>3 (0.6%)</b> |

### DISCUSSION

The conventional L-J medium preparation was adopted from National Tuberculosis and Leprosy control program (NTBLCP 2013), Laboratory manual. One challenge facing isolation of MTB was the growth duration on artificial media that takes between 21 -60 days. This research worked on modifying Lowenstein –Jensen medium for faster mycobacterial growth.

The modified L-J medium contained potato flour in the first attempts of the modification. At the lowest concentration of egg (3%) MTB grew in the medium in 78

days while in at egg concentrations of 70% and 75% MTB grew in 17 days. The presence of sodium citrate, high concentration of egg, purified and liquefied sputum contribute immensely to the earlier isolation of MTB with modified L-J (Rath *et al* 2013; Sophie *et al*, 2019). The improved L-J was used to carry out drug sensitivity test of the isolated MTB by proportion method, it grew on the 5<sup>th</sup> day unlike others that took longer days (Rajani *et al*, 2013). Because MTB was isolated in 5 days from modified L-J, both culture and sensitivity of patients' samples can be achieved in 14 days. *Mycobacterium tuberculosis* grows on

potato L-J medium but it does better on modified L-J medium without potato flour.

The differentiation of *Mycobacterium tuberculosis* complex (MTBC) from non-MTBC using TBAgMPT<sub>64</sub> test kit is consistent with the reports of Ryan, Barha and Putra (Ryan *et al* 2014; Barha and Putra, 2019). In addition the purification of sputum samples with 4% freshly prepared sodium hydroxide (NaOH), 69 µL/100 mL ciprofloxacin, 69 µL/100 mL gentamycin, 69 µL/100 mL azithromycin, 69 µL/100 mL nalidixic acid, 69 µL/100 mL amoxiallin, 69 µL/100 mL difflucan were added to the modified L-J to ward off contaminants (Burns and Rolain, 2014; Clair *et al*, 2016). MTB/Rifampicin resistant tests to find resistant mycobacteria were carried out with GeneXpert method (Vivek *et al*, 2020). This work adopted the purification of sputum samples prescribed by Giovanni *et al*, (2013)

Catherine and her team used Vitamin C to boost the effects of anti-TB in their work (Catherine *et al*, 2013; Gaglani *et al*, 2023).

Anti-TB drug sensitivity approaches used were directly and proportional methods. They align with Rajani *et al*.(2013) methods. The results were consistent and were achieved within 10-14 days from the first inoculation of MTB.

To identify the MTB growth early from the culture, use can be made of magnifying lens. This aligns with the work of Ramzi and Dedier.(2014).

### Conclusion and Suggestion

We concluded that this research demonstrated early isolation, differentiation and identification of Mycobacteria isolated from this work.

The modified L-J was found very helpful in achieving this success.

Therefore, these new approaches are recommended for the routine TB diagnosis as well as in TB research programmes.

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# Molecular Detection of Merozoite Surface Protein 1 and 2 in Plasmodium Falciparum Infected Subjects in Some Selected Hospitals, Bayelsa State, Nigeria

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## ABSTRACT

**Abstract:** Malaria still remains a major burden, despite efforts in curbing this disease in Bayelsa state. To better manage and treat this disturbing disease, it is essential to detect merozoite surface protein 1 (msp1) and merozoite surface protein 2 (msp2) in *Plasmodium falciparum* infected subjects. This present study was aimed to detect msp1 and msp2 in *Plasmodium falciparum* infected subjects in Bayelsa state. A cross sectional design was adopted. The parasite's DNA was extracted using kit method. A total of 50 positive malaria subjects with parasite density of  $\geq 5,000/\mu\text{l}$  were selected for this study. However, samples with positive *P.falciparum* examined by microscopy were confirmed with the polymerase chain reaction technique. The msp1 and msp2 genes were amplified with Nested-Touch Dawn PCR method and their amplicons analyzed by electrophoresis on a 1% agarose gel stained with 5  $\mu\text{l}$  Safe-View dye. The alleles present were resolved according to their molecular ladder under a blue light. Forty percent (40.0%) of the positive samples were confirmed as *P.falciparum* by PCR. In msp1, RO33 and MAD20 alleles were detected of which, 10% RO33 alleles was predominant followed by 5.0% MAD20 alleles. In msp2, no alleles were observed. The association between multiplicity of infection and parasite density was -13 ( $P < 0.05$ ). This study detected only MSP1 in subjects being infected with *P.falciparum*. Drugs for the treatment of malaria should be design to target MSP1 strains of *Plasmodium falciparum* circulating in Bayelsa state.

**Key words:** Molecular Detection, Merozoite Surface Protein, *Plasmodium falciparum*, Infected Subjects, Malaria Parasitaemia

## INTRODUCTION

Despite all the efforts by both governmental and non-governmental organizations to control malaria, it is still a global concern in respect to morbidity and mortality [1]. In line with the emergence of new strains of *Plasmodia*, *Plasmodium* strains resistant to Artemisinin Based Combination Therapy (ACT) emerging and the emergence of resistant vectors to insecticides is a major contributory factor to the endemicity of malaria parasites [2]. Five *Plasmodium* species are known to cause malaria in humans [3], such as the *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium knowlesi* [4]-[6].

Malaria, a tropical disease is caused by *Plasmodium* species and mainly acquired through the bite of an infected female anopheles mosquito [7], [8]. Among all the *Plasmodium* species, *Plasmodium falciparum* is the deadliest, caused from mild to severe malaria conditions associated with morbidity and mortality. Additionally, it is frequently accompanied with antimalarial drug resistance and failed vaccines trials [9].

It had been reported that genetic variety of *Plasmodium falciparum* plays an imperative role in defining the power in the spread of malaria. However, numerous *Plasmodium falciparum* genes had shown extensive genetic polymorphism in merozoite surface proteins 1 (msp1) and merozoite surface proteins 2 (msp2) in different

environmental sites where malaria is endemic. In addition, msp1 and msp2 genes are extensively used genes to study the allelic multiplicity and incidence of *Plasmodium falciparum* [10]. These genetic markers are essential molecular tool used in detecting malaria parasites [11], [12]. However, merozoite surface protein 2 (MSP2) plays some vital roles as major vaccine candidates while merozoite surface protein 1 (MSP1) facilitates the invasion of red blood cells in the human host [13].

It is commonly reported that diagnosis of malaria is routinely examine with Giemsa stained blood film under light microscope [3]. However, malaria parasites (*Plasmodium falciparum* for instance) is better and more efficiently detected with the polymerase chain reaction (PCR) by amplifying the genes coding for msp1 and msp2 [14]. Therefore, the interest in this present study is geared towards detecting msp1 and msp2 in *Plasmodium falciparum* infected subjects in some selected hospitals in Bayelsa state.

## MATERIALS AND METHOD

### Study Design

A cross-sectional observational study design was adopted. A cross-sectional observational study design was on *Plasmodium* infected subjects with malaria parasites density  $\geq 5000$  parasites/ $\mu\text{l}$  of blood at Molecular



Laboratory Unit in the Medical Laboratory Science Department, Niger Delta University, Bayelsa state.

#### Study Site

The study was carried out in Bayelsa state, Nigeria. There are eight local government in Bayelsa state, with its capital situated in Yenagoa whereas the state is divided into three federal senatorial districts. This senatorial district includes: The Central that comprising of Yenagoa, Kulokumo-Opukuma and Southern Ijaw Local Government Areas, West senatorial district comprising of Brass, Nembe and

Ogbia Local Government Areas, while East senatorial district comprising of Sagbama and Ekeremor Local Government Areas respectively as shown in [figure 1].

Accordingly, the 2006 census stated that Bayelsa state has a population of approximately 1.7million people. It is geographically located latitude 4°15'1north, 5°23'1south and longitude 5°22'1west, 6°45'1east. Also, bounded with Delta state on the north, Rivers state on the east and the Atlantic Ocean on the west and south [16].

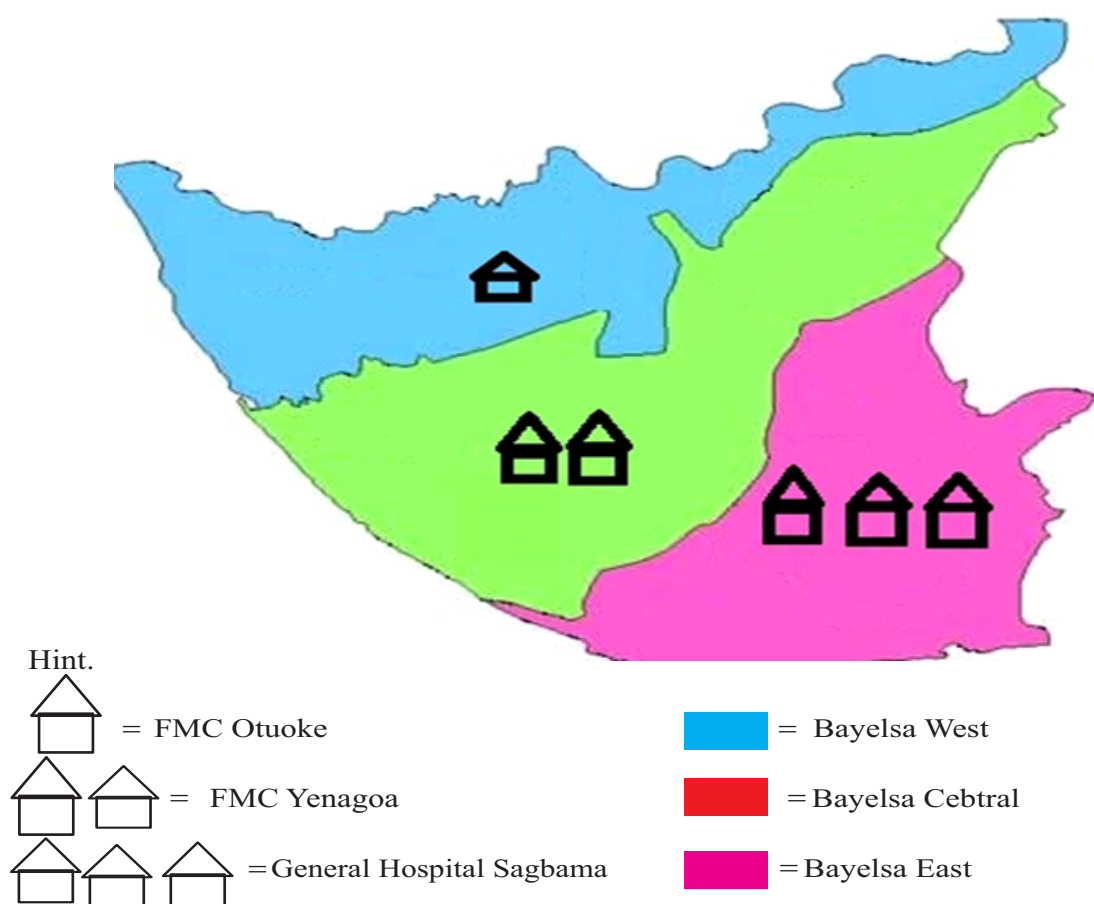


Figure 3.2. Map of Bayelsa State

#### Study population:

The population of this study included all subjects that were diagnosed with *Plasmodium falciparum* malaria by microscopy and haven parasitaemia levels of  $\geq 5000$  parasites/ $\mu$ l in the selected hospitals in Bayelsa state.

**Inclusion criteria:** The followings were the inclusion criteria used for this work.

1. Subjects from the ages of six (6) months old visiting the laboratory with fever or physician clinical diagnosis of malaria
2. Subjects from the ages of six (6) months old visiting the laboratory with complaint of fever
3. Subjects from the ages of six (6) months old visiting the laboratory with absence of documented malaria treatment in the past two weeks prior to this work.
4. Only subjects confirmed infected with *Plasmodium falciparum* with parasite density of

$\geq 5,000$  parasites/ $\mu$ l from examination of Giemsa stained blood film were used for molecular analysis.

#### Exclusion criteria:

The exclusion criteria for this present study were:

1. Subjects from ages of six (6) months old visiting the laboratory who refused granting consent
2. Subjects from ages of six (6) months old visiting the laboratory noted of having evidence of any documented adverse drug effect.

#### Malaria Parasite Detection

Thick and thin blood films were prepared, stained with a 10% Giemsa working solution and examined with  $\times 100$  objective lens under a light microscope. Parasites detected were counted simultaneously with differential white blood cells, and parasites density were calculated using the formula given below:



$$\text{Malaria Parasitaemia} = \frac{\text{Parasite counted}}{200\text{WBC}} \times 8000/\mu\text{l}$$

Malaria parasitaemia levels were quantified as < 5000 parasites/μl indicating a low parasitaemia while ≥ 5,000 parasites/μl of blood as high parasitaemia.

#### DNA Extraction

Parasites DNA were extracted using the kit extraction method. Approximately, 400μl of genomic lysis was added to 100μl of blood, mixed, vortexed for 5 seconds and incubated at room temperature for 10 minutes. The mixture was transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10000x g for 1 minute. The collection tube along with their flow through were discarded. The Zymo-Spin IIC column of the mixture was transferred to a new collection tube. A 200μl DNA Prewash Buffer was added to the spin column and centrifuged at 10 000xg for 1 minute. A 500μl of g-DNA wash buffer was added to the spin column, centrifuged again at 10000x-g for 1 minute. The spun column was transferred into another clean microcentrifuge tube, 50μl of DNA elution buffer was added to the column, incubated at room temperature for 5 minutes and centrifuged at top speed for 30 seconds to elute the DNA. The eluted DNA was tested for its purity, quantity of the content and stored at 5°C-20°C for further analysis.

#### DNA Purification and Quantification

The purity and the quantity of the DNA was measured using Nano-drop 1000 Spectrophotometer. Using software appt, it was launched by clicking the Nano-drop icon twice while the equipment was initialized with 2μl of sterile distilled water and blanked with normal saline. 2μl of each extracted DNA was loaded separately onto lower pedestal, the upper

pedestal was brought down to make contact with the extracted DNA placed on the lower pedestal. The quantity and purity of the DNA were read at interval with a corresponding software. The ratio of absorbance (260/280) was used to determine the DNA purity as previously adopted [17] - [18], [9].

#### Molecular Detection of *Plasmodium falciparum*

Polymerase Chain Reaction was used to confirm malaria positive samples examined with the microscopic Giemsa stained blood films. *P.falciparum* genes as shown in (Table i) was used. The reaction was performed in a final volume of 30μl containing: 2X Master-Mix, water, extracted *P.falciparum* DNA template, forward and reverse primers according to the manufacturer's instructions.

The reaction was performed with 30μl containing 2X Master-Mix and water, amplicon from the first PCR product as DNA template, forward and reverse primers. In these reactions, 1μl each of the first PCR product were added as the DNA templates. Each amplification conditions were set as follows: For msp1, it was set at 98°C for 5min, 95°C for 40s, 75°C for 1min, 70°C for 1min, 68°C for 40secs, 69°C for 5mins and a holding period at 72°C for 10mins in 30 cycles while msp2 condition was 98°C for 5min, 95°C for 40s, 75°C for 1min, 72°C for 1min, 68°C for 40secs, 69°C for 5mins and a holding period at 72°C for 10mins in 30 cycles respectively. While their PCR results were resolved using agarose gel electrophoresis [19]. The prevalence of each

**Table i: Primer Sequence used for the amplification of *Plasmodium falciparum* and their Length**

| Species             | Primer Name | Primer Sequence      | Primer Length |
|---------------------|-------------|----------------------|---------------|
| <i>P.falciparum</i> | Pf-OF       | AAAAGGAGAAGAAAAAATAA | 21            |
|                     | Pf-OR       | AAAATAAACAAAATCATC   | 18            |

PCR Amplification: In this reaction, a 2μl of the extracted DNA was added to the PCR component. The PCR condition was set at 98°C for 5min, 95°C for 40s, 75°C for 1min, 70°C for 1min, 68°C for 40secs, 69°C for 5mins and a holding period at 72°C for 10mins in 30 cycles. The result PCR was resolved using agarose gel electrophoresis [19]. A nested touch down PCR technique was used to detect the *P.falciparum* msp1, *P.falciparum* msp2 genes. Specific primers designed as shown in (Table ii) were used to amplify msp1 and msp2 genes in the primary reactions.

**Table ii: Primer sequence and length**

| Gene | PCR Round | Primer Name | Primer Sequence (5-3)          | Primer Length |
|------|-----------|-------------|--------------------------------|---------------|
| MSP1 | Primary   | M1-OR       | CTAAAAGCTTTAGAAGATGCAGTATTG    | 27            |
|      |           | M1-OR       | CTTAAATAGTATTCTAATTCAAGTGGATCA | 30            |
|      | Secondary | M1-MF       | AAATGAAGGAACAAGTGAACAGCTGTTAC  | 30            |
|      |           | M1-MR       | ATTCCAAGGATTTGTACGTCTTGAATTACT | 30            |
|      |           | M1-KF       | AATGAAGAAGAAATTACTACAAAAGGTGC  | 29            |
|      |           | M1-KR       | GCTTGCATCAGCTGGAGGGCTGCACCAGA  | 30            |
|      |           | M1-RF       | TAAGGGATGGAGCAAATACTCAAGTTGTGT | 30            |
|      |           | M1-RR       | CATCTGAAGGATTTCGAGCACCTGGACATC | 30            |
|      | Primary   | MS2-1       | ATGAAGGTAATTAACATTGTCTATTATA   | 30            |
|      |           | MSP2-4      | ATATGGCAAAAGATAAAACAAGTGTGCTG  | 30            |
|      | Secondary | 3D7-A1      | GCTAGAAAGTAAGCCTTCCTACTGGT     | 27            |
|      |           | 3D7-A2      | CATTTGTTTCGGCATTATTATGA        | 23            |
|      |           | FC27-B1     | GCAAATGAAGGTTCTAATACTAATAG     | 26            |
|      |           | FC27-B2     | GCTTTGGGTCCTTCTCAGTTGATTC      | 26            |

allelic type was determined as the presence of PCR products for the type in the total number of amplified bands for the corresponding locus. A nested touch down PCR technique was used to detect the *P.falciparum* MSP1, *P.falciparum*

MSP2 genes. Specific primers designed as shown in (Table ii) were used to amplify the allelic families in the secondary reactions. The reactions were performed in a final volume of 30μl containing: 2X Master-Mix, water,

amplicon from the first PVR product as DNA template, forward and reverse primers. In these secondary reactions, 1µl each of the first PCR product were added as DNA templates according to the manufacturer instructions. In msp1, each amplification conditions were set as follows: the MAD20 families were set at 98°C for 5min, 95°C for 40s, 83°C for 1min, 78°C for 1min, 68°C for 40secs, 69°C for 5mins and a holding period at 72°C for 10mins in 30 cycles; K1 allelic families were at 98°C for 5min, 95°C for 40s, 86°C for 1min, 81°C for 1min, 68°C for 40secs, 69°C for 5mins and a holding period at 72°C for 10mins in 30 cycles and the RO33 allelic families were set at 98°C for 5min, 95°C for 40s, 86°C for 1min, 81°C for 1min, 68°C for 40secs, 69°C for 5mins and a holding period at 72°C for 10mins in 30 cycles [19]. In msp2, each amplification conditions were set as follows: the 3D7 allelic families were set at 98°C for 5min, 95°C for 40s, 70°C for 1min, 65°C for 1min, 68°C for 40secs, 69°C for 5mins and a holding period at 72°C for 10mins in 30 cycles and FC27 allelic families were at 98°C for 5min, 95°C for 40s, 72°C for 1min, 67°C for 1min, 68°C for 40secs, 69°C for 5mins and a holding period at 72°C for 10mins in 30 cycles [19].

### DNA Electrophoresis

The PCR products were resolved and sized against a 100bp molecular weight marker on a 1% agarose gel made in a 1X Tri-Boris EDTA containing 5µl Safe-View and ran in a horizontal tank filled with 1X Tri-Boris EDTA for 30mins at 350V. All gels were visualized under a blue light transillumination and the electrophoretic condition was 100 mV for 30 min. The size and fragment of the respective bands were estimated by comparing bands with a 100kb plus DNA ladder [19].

### Multiplicity of Infection

The Multiplicity of Infection (MOI) or number of genotypes per infection was calculated by dividing the total number of fragments detected in msp1 and msp2 by the number of samples positive for the same marker [9]. Polyclonal infections were more in majority of the patients for msp1 allelic families [20].

### Data Analysis

All data obtained from the results of the Giemsa stained blood films were entered into Statistical Package for the Social Science (SPSS) version 23 and analyzed using analyzed using descriptive statistics for the categorical variables, while an inferential was used for continuous variables. The allelic frequency of msp1 and msp2 was calculated as proportion of the allele detected for each of the

allelic family from the overall total alleles detected. The frequency of polyclonal infection was calculated using number of samples with more than one amplified fragment out of the total samples. Multiplicity of infection was determined by dividing the total number of alleles detected in msp1 and msp2 by the total number of positive samples for both genetic marker. Descriptive statistics was used to calculated mean standard deviation of the malaria parasitaemia, and results were expressed in percentages and presented in frequencies. While Spearman rank correlation coefficient (r), was used to determine the probable associations exiting between the parasitaemia level and multiplicity of infection. Statistical significance was defined as  $p \leq 0.05$

## RESULTS

Table 1a showed the distribution of malaria parasitaemia among the *Plasmodium falciparum* infected subjects in some selected hospitals Bayelsa state, and level of parasitaemia varied between  $< 5000$  parasites/µl to  $\geq 15,000$  parasites/µl of blood. Of the 180 positive cases, 130(72.2%) had malaria parasitaemia less than 5000 parasites/µl of blood and 50(27.8%) had malaria parasitaemia of 5000 parasites/µl of blood and above. Out of the 50 subjects with higher malaria parasitaemia density, 22(12.2%) had parasitaemia of 5000-10000 parasites/µl of blood, 21(11.7%) had parasitaemia of 10001-15000 parasites/µl of blood and 7(3.9%) had parasitaemia above 15000 parasites/µl of blood

Prevalence of malaria parasitaemia based on gender of subjects were 65.9% of which, 72.2% had malaria parasitaemia of  $>5000$  parasite/µl (consisting 62 males and 68 females) while 50(27.8%) subjects malaria parasitaemia of  $\geq 5000$  parasite/µl. Of this, 16.7% of subjects had parasitaemia level between 5000-10000 parasite/µl of which, 12% males and 1.8% females, followed by 8.3% of subjects had parasitaemia level between 10001-15000 parasite/µl of which, 6% males and 10% females and 16.7% of subjects had highest parasitaemia level of  $>15000$  parasite/µl of which, 7.2% males and 8.6% females respectively.

Table 2 and plate 1a showed PCR distribution of *Plasmodium falciparum* by Age and Gender. Parasite genomic DNA was extracted from the 50 blood samples with the highest parasitaemia levels  $\geq 5000$  parasites/µl of blood of which, 20(40.00%) *P.falciparum* were confirmed by PCR. Of this number, 8(38.1%) were from males while 12(41.4%) females. In males: eight (8) malaria parasites were examined from patients below 11 years old of which, 3(37.5%) *P.falciparum* were confirmed. Five (5) malaria

**Table 1a: Prevalence of Malaria Parasitaemia by Age using Microscopy**

| Age (Yrs.) | Parasitaemia (parasite/µl) |            |             |          | Total     |
|------------|----------------------------|------------|-------------|----------|-----------|
|            | $<5000$                    | 5000-10000 | 10001-15000 | $>15000$ |           |
| $<11$      | 39(70.9%)                  | 9(16.4%)   | 6(10.9%)    | 1(1.8%)  | 55(30.6%) |
| 11-20      | 23(74.2%)                  | 1(3.2%)    | 5(16.1%)    | 2(6.5%)  | 31(17.2%) |
| 21-30      | 28(90.3%)                  | 2(6.5%)    | 1(3.2%)     | 0        | 31(17.2%) |
| 31-40      | 20(69.0%)                  | 4(13.8%)   | 3(10.3%)    | 2(6.9%)  | 29(16.1%) |
| 41-50      | 10(52.6%)                  | 3(15.8%)   | 4(21.1%)    | 2(10.5%) | 19(10.6%) |
| 51-60      | 6(66.7%)                   | 1(11.1%)   | 2(22.2%)    | 0        | 9(5.0%)   |
| 61-70      | 4(100.0%)                  | 0          | 0           | 0        | 4(2.2%)   |
| $\geq 70$  | 0                          | 2(100.0%)  | 0           | 0        | 2(1.1%)   |
|            | 130(72.2%)                 | 22(12.2%)  | 21(11.7%)   | 7(3.9%)  | 65.9%     |

The prevalence of malaria parasitaemia based on age of the subjects were 65.9% of which, 130(72.2%) of subjects had malaria parasitaemia of  $>5000$  parasite/µl while 50(27.8%) subjects malaria parasitaemia of  $\geq 5000$  parasite/µl.

Table 1b showed the distribution of malaria parasitaemia (parasite density) among *Plasmodium falciparum* infected patients by gender in some selected hospitals Bayelsa state. Of the 180 malaria positive patients, 130(72.2%) had parasite density of less than 5000 parasites/μl of blood while 50(27.8%) had parasite density of 5000 and above parasites/μl of blood. Of the 50 malaria positive patients with parasite density of 5000 and above parasites/μl of blood, 21(16.7%) had parasite density between 5000-10000 parasites/μl of blood, 15(8.3%) had parasite density between 10001-15000 parasites/μl of blood and 14(7.8%) had parasite density above 15000 parasites/μl of blood.

**Table 1b: Prevalence of Malaria Parasitaemia by Gender by Microscopy**

| Gender  | Parasitaemia (parasite/μl) |            |             |          | Total      |
|---------|----------------------------|------------|-------------|----------|------------|
|         | <5000                      | 5000-10000 | 10001-15000 | >15000   |            |
| Males   | 62(74.7%)                  | 10(12.0%)  | 5(6.0%)     | 6(7.2%)  | 83(46.1%)  |
| Females | 68(70.1%)                  | 11(1.8%)   | 10(10.8%)   | 8(8.6%)  | 97(53.9%)  |
| Total   | 130(72.2%)                 | 21(16.7%)  | 15(8.3%)    | 14(7.8%) | 180(65.9%) |

parasites were examined from patients between the ages of 11-20 years of which, 2(40.0%) *P.falciparum* were confirmed. Three (3) malaria parasites examined from patients between the ages of 31-40 years of which, 1(33.3%) *P.falciparum* were confirmed and four (4) malaria parasites were examined from patients between the ages of 41-50 years of which, 2(50.0%) *P.falciparum* were confirmed. In females: nine (9) malaria parasites were examined from patients below 11 years old of which, 7(77.8%) *P.falciparum* were confirmed. Two (2) malaria parasites were examined from patients between the ages of 11-20 years of which, 1(50.0%) *P.falciparum* were confirmed. Six (6) malaria parasites examined from patients between the ages of 31-40 years of which, 1(16.7%) *P.falciparum* were confirmed. Five (5) malaria parasites were examined from patients between the ages of 41-50 years of which, 2(40.0%) *P.falciparum* were confirmed and two (2) malaria parasites were examined from patients over the ages of 70 years of which, 1(50.0%) *P.falciparum* were confirmed respectively.

## Discussion

Of the 50 samples taken for PCR with malaria parasitaemia  $\geq 5000/\mu\text{l}$  of blood, 20(40.00%) showed amplification while 30(60.0%) showed no amplification for *P.falciparum* respectively. Reason could possibly be due to specificity and sensitivity of the choice of primers used. This finding was in agreement with other study [9], but lower in similar studies [21]. This finding is also in conformity with a previously reported case of importance of molecular genotyping of *P.falciparum* [14]. However, genotyping of *Plasmodium falciparum* msp1 and msp2 are recommended for the antimalaria clinical trials as standard markers to distinguish recrudescence from a newly infecting malaria episode [22]. Also, to the best of our knowledge, this kind of study investigating the genetic diversity of msp1 and msp2 in malaria parasites circulating hospitals in Bayelsa state. Hence, our study aimed to detect msp1 and msp2 among *Plasmodium falciparum* infected subjects in hospitals, Bayelsa state is scarcity.

Successfully, this study detects only the msp1 gene in *Plasmodium falciparum* infected subjects. This might suggest that only this strain of the parasite seems to be

**Table 2.: PCR Distribution of *P. falciparum* Infection by Age and Gender**

| Age (Yrs.) | Male |          | Female |           |
|------------|------|----------|--------|-----------|
|            | NE   | NI       | NE     | NI        |
| <11        | 8    | 3(37.5%) | 9      | 7(77.8%)  |
| 11-20      | 5    | 2(40.0%) | 2      | 1(50.0%)  |
| 21-30      | 0    | 0        | 4      | 0         |
| 31-40      | 3    | 1(33.3%) | 6      | 1(16.7%)  |
| 41-50      | 4    | 2(50.0%) | 5      | 2(40.0%)  |
| 51-60      | 0    | 0        | 1      | 0         |
| 61-70      | 0    | 0        | 0      | 0         |
| >70        | 1    | 0        | 2      | 1(50.0%)  |
|            | 21   | 8(38.1%) | 29     | 12(41.4%) |

Key: (NE) = Number Examined, (NI) = Number Infected, (%) = Percentage, < = Less than and > = Greater than

Of the 20 *P.falciparum* from patients were detected for msp1 and msp2 by age using nested touch down PCR, 2(10.0%) msp1 and 0(0.0%) msp2 were detected in patients within the aged of 40 years and above. In msp1, 1(20.0%) MAD20 allelic, 0(0.0%) K1 allelic and 2(40.0%) R033 allelic families were detected. Polyclonal infections were detected in 2(10.0%) MAD20/R033 while monoclonal infections were detected in 1(5.0%) MAD21 of msp1 respectively. In MSP2, 0(0.0%) 3D7 allelic and 0(0.0%) FC27 allelic families were detected. Polyclonal infections were detected in 0(0.0%) and monoclonal infections were detected in 0(0.0%) of the allelic families respectively as shown in (Table 3a).

**Table 3a: Distribution of MSP1 and MSP2 by Age by PCR Technique**

| Age (yrs.) | NE | MSP1     |    |          | MSP2 |      |
|------------|----|----------|----|----------|------|------|
|            |    | MAD20    | K1 | R033     | 3D7  | FC27 |
|            |    | 0        | 0  | 0        | 0    | 0    |
| 21-40      | 2  | 0        | 0  | 0        | 0    | 0    |
| >40        | 5  | 1(20.0%) | 0  | 2(40.0%) | 0    | 0    |
|            | 20 | 1(5.0%)  | 0  | 2(10.0%) | 0    | 0    |

Key: (yrs.) = Years, (NE) = Number Examined, (MSP1) = Merozoite Surface Protein 1, (MSP2) = Merozoite Surface Protein 2, (MAD20) = MSP1: MAD20 Alleles in *Plasmodium falciparum*, (K1) = MSP1: K1 Alleles in *Plasmodium falciparum*, (R033) = MSP1: R033 Alleles in *Plasmodium falciparum*, (3D7) = MSP2: 3D7 Alleles in *Plasmodium falciparum*, (FC27) = MSP1: FC27 Alleles in *Plasmodium falciparum*, (%) = Percentage, ( $\leq$ ) = Less than or Equal to and ( $\geq$ ) = Greater than or Equal to

A total of 20 *P.falciparum* from patients were detected for msp1 and msp2 by gender using nested touch down PCR of which, 2(10.0%) msp1 genes and 0(0.0%) msp2 genes were detected. For MSP1, 0(0.0%)MAD20, 0(0.0%)K1 and 0(0.0%)R033 alleles were detected in males while 1(5.0%)MAD20 alleles, 0(0.0%)K1 alleles and

2(40.0%)R033 alleles in females. A polyclonal infection was detected as 2(10.0%)MAD20/R033 and monoclonal infection as 1(5.0%)MAD21 in MSP1. For MSP2, 0(0.0%)3D7 and 0(0.0%)FC27 alleles were detected in males and females respectively as shown in (Table 3b and Plate 1b-f).

**Table 3b: Distribution of MSP1 and MSP2 by Gender by PCR Technique**

| Gender | n=20 | MSP1    |    |          | MSP2 |      |
|--------|------|---------|----|----------|------|------|
|        |      | MAD20   | K1 | R033     | 3D7  | FC27 |
| Male   |      | 0       | 0  | 0        | 0    | 0    |
| Female |      | 1(3.3%) | 0  | 2(66.7%) | 0    | 0    |
|        |      | 1(5.0%) | 0  | 2(10.0%) | 0    | 0    |

Key: (yrs.) = Years, (NE) = Number Examined, (MSP1) = Merozoite Surface Protein 1, (MSP2) = Merozoite Surface Protein 2, (MAD20) = MSP1: MAD20 Alleles in *Plasmodium falciparum*, (K1) = MSP1: K1 Alleles in *Plasmodium falciparum*, (R033) = MSP1: R033 Alleles in *Plasmodium falciparum*, (3D7) = MSP2: 3D7 Alleles in *Plasmodium falciparum*, (FC27) = MSP1: FC27 Alleles in *Plasmodium falciparum* and (%) = Percentage.

Table 4 showed the alleles determined by their molecular size and family type. In msp1, 1(5.0%) MAD20 allelic, 0(0.0%) K1 allelic and 2(10.0%) R033 allelic families were detected. Polyclonal infections were detected in 2(10.0%) MAD20/R033 while monoclonal infections were detected in 1(5.0%) MAD21 of msp1 in females. While 0(0.0%)

MAD20 allelic, 0(0.0%) K1 allelic and 0(00.0%) R033 allelic families were detected. Polyclonal infections were detected in 2(10.0%) MAD20/R033 while monoclonal infections were detected in 0(0.0%) MAD20 of MSP1 in males and the multiplicity of infection of the alleles was 0.15

**Table 4: Multiplicity of Infection in MSP1 and MSP2 by Age**

| Age (yrs.) | Total | MOI  | MSP1    |    |          | Total | MOI | MSP2 |      |
|------------|-------|------|---------|----|----------|-------|-----|------|------|
|            |       |      | MAD20   | K1 | R033     |       |     | 3D7  | FC27 |
| <20        | 20    | 0    | 0       | 0  | 0        | 20    | 0   | 0    | 0    |
| 20-40      | 20    | 0    | 0       | 0  | 0        | 20    | 0   | 0    | 0    |
| >40        | 20    | 0.15 | 1(5.0%) | 0  | 2(10.0%) | 20    | 0   | 0    | 0    |
|            |       | 0.15 | 1(5.0%) | 0  | 2(10.0%) |       | 0   | 0    | 0    |

Key: MOI = Multiplicity of Infection, (MSP1) = Merozoite Surface Protein 1, (MSP2) = Merozoite Surface Protein 2, (MAD20) = MSP1: MAD20 Alleles in *Plasmodium falciparum*, (K1) = MSP1: K1 Alleles in *Plasmodium falciparum*, (R033) = MSP1: R033 Alleles in *Plasmodium falciparum*, (3D7) = MSP2: 3D7 Alleles in *Plasmodium falciparum*, (FC27) = MSP1: FC27 Alleles in *Plasmodium falciparum* and (%) = Percentage.

In total, 15.0%(2/20) and 0% of the parasite strains contained multiplicity of infections (MOIs) on msp1 and msp2 respectively. The MOIs for both msp1 and msp2 are summarized in table 5. The MOI for msp1 was 0.15 with a range of 1-2 strains and was higher than MOI for msp2 of 0 with zero range of strains. There is no significance association existing between malaria parasitaemia and multiplicity of infections in *P.falciparum* infected subjects

in hospitals in Bayelsa state. The Spearman's correlation coefficient (r) was - 0.13 at a mean  $\pm$  SD of  $3112.63 \pm 10256.00$  parasites/ $\mu$ l of blood ( $p = 0.37$ ) as in table 8. The mean  $\pm$  SD of malaria parasitaemia was  $3112.63 \pm 10256.00$  and multiplicity of *P.falciparum* infection was 0.15, association (r) between malaria parasitaemia and multiplicity of infection was -0.13 at P-value of 0.37

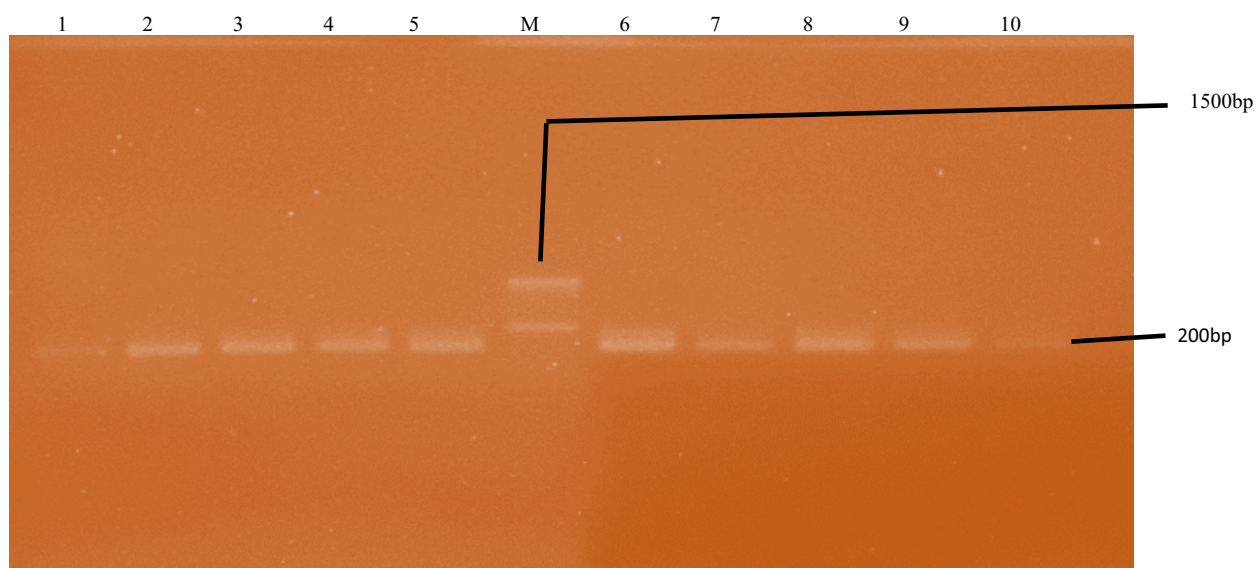
**Table 5: Association between Parasitaemia and Multiplicity of Infection in P.falciparum**

| Parasitaemia                       |                        | Multiplicity of Infection |      |
|------------------------------------|------------------------|---------------------------|------|
| Mean $\pm$ SD (parasites/ $\mu$ l) | 3112.63 $\pm$ 10256.00 | n                         | 20   |
|                                    |                        | r                         | -.13 |
|                                    |                        | p                         | .37  |

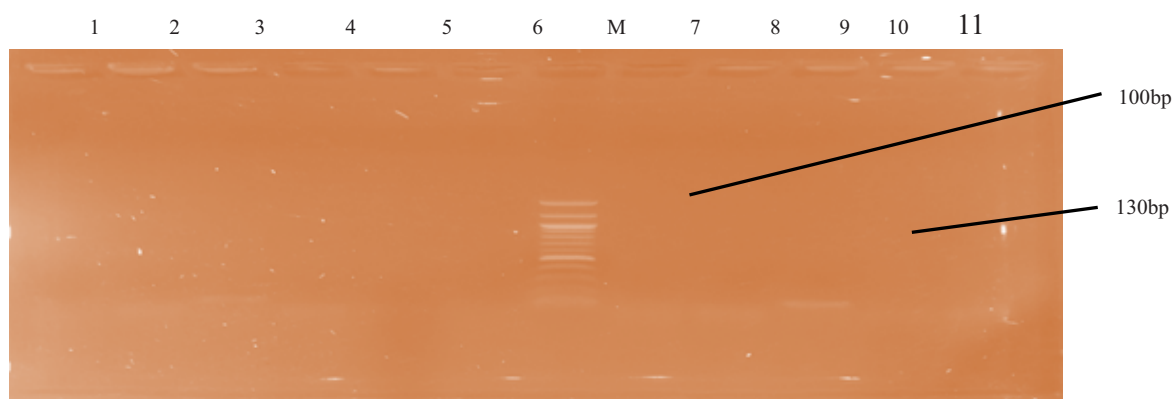
Key: n = Number of patients, r = Spearman's correlation coefficient, p = error probability, SD = standard deviation, Multiplicity of Infection Ratio = Alleles MAD20/R033



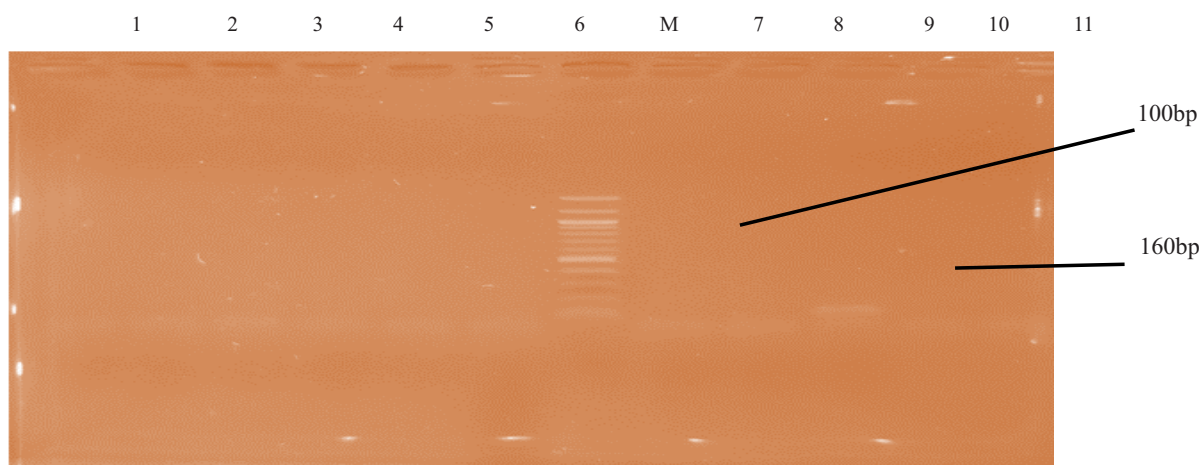
# *Molecular Detection of Merozoite Surface Protein*



**Plate 1a:** Agarose gel electrophoresis of *Plasmodium falciparum* gene in *P. falciparum* infected patients in some selected hospital in Bayelsa state. Lane 1-10 represents the *Plasmodium falciparum* gene band (200bp). Lane M represents the 1500bp Molecular ladder of 1500bp. bp = base pair

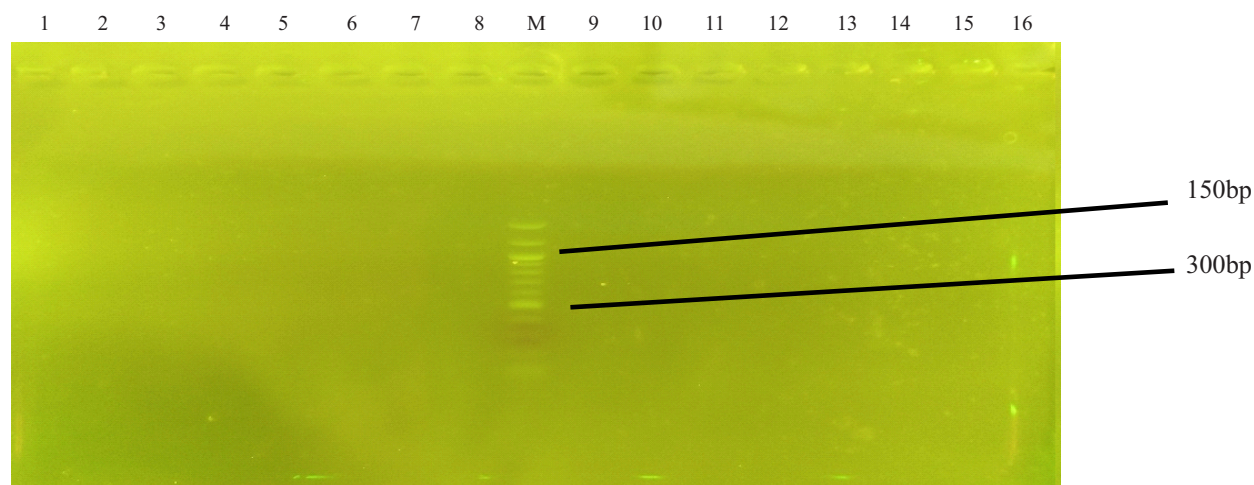


**Plate 1b:** Agarose Gel Electrophoresis of MSP1-RO33 Gene of some selected *P. falciparum* in *P. falciparum* infected patients in some selected hospital in Bayelsa state. Lane 3 and 9 represents the MSP1-RO33 gene band (130bp). Lane M is a 1000bp Molecular ladder of 1500bp. bp = base pair

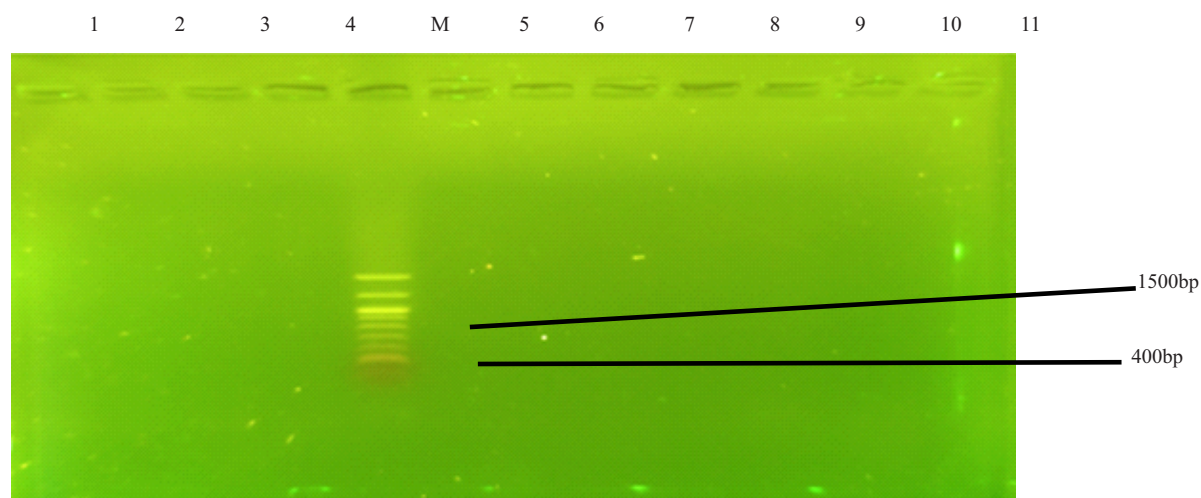


**Plate 1c:** Agarose Gel Electrophoresis of MSP1-MAD20 Gene variant of some selected *P. falciparum* in *P. falciparum* infected patients in some selected hospital in Bayelsa state. Lane 9 represents the MSP1-MAD20 gene band (160bp). Lane M represents the 1000bp Molecular ladder of 1500bp. bp = base pair

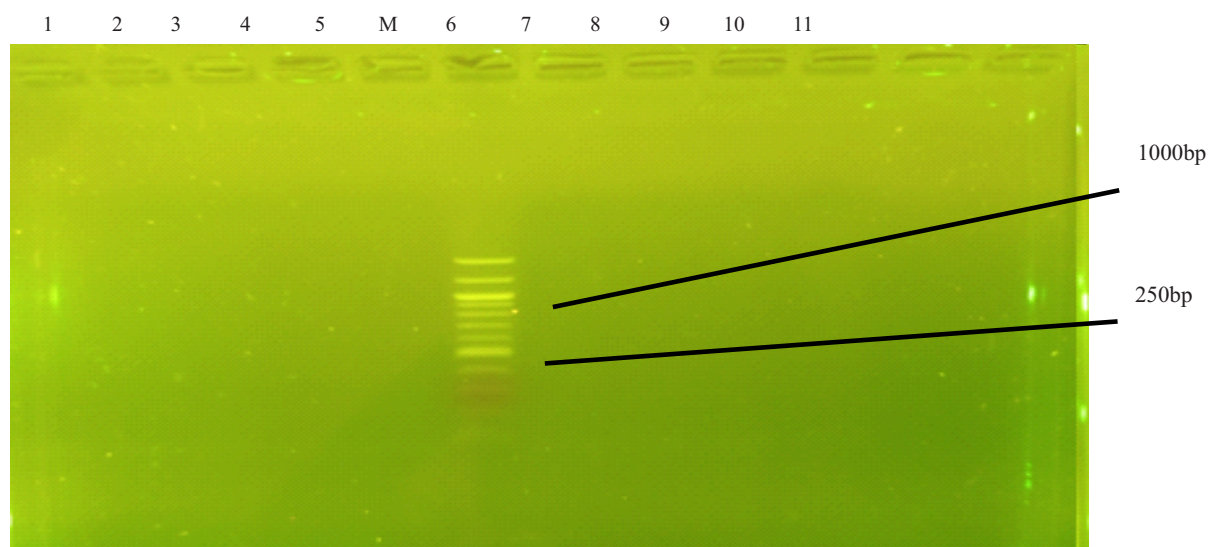




**Plate 1d:** Agarose Gel electrophoresis MSP1-K1 Gene of some selected *P. falciparum* in *P. falciparum* infected patients in some selected hospital in Bayelsa state. Lane 1-15 represents the negative results of MSP1-K1 gene band (300bp). Lane M represents the 1500bp Molecular ladder of 1500bp. bp = base pair



**Plate 1e:** Agarose Gel Electrophoresis of MSP2-3D7 Gene of some selected *P. falciparum* in *P. falciparum* infected patients in some selected hospital in Bayelsa state. Lane 1-11 represents the negative result of MSP2-3D7 gene band (400bp). Lane M represents the 1500bp Molecular ladder of 1500bp.



**Plate 1f:** Agarose Gel Electrophoresis of MSP2-FC27 Gene of some selected *P. falciparum* in *P. falciparum* infected patients in some selected hospital in Bayelsa state. Lane 1-11 represents the negative result of MSP2-FC27 gene band (250bp). Lane M represents the 100bp Molecular ladder of 1500bp. bp = base pair

predominant in the state. This finding conformed but slightly differ with the previous studies done [9]. Genetic polymorphism the merozoite surface proteins of *Plasmodium falciparum* detected were analyzed demonstrated allelic specific PCR in the genotyping msp1 showed the presence of R033 and MAD20 allelic families in the *Plasmodium falciparum*. Of the two allelic families of msp1, R033 was the most predominant, followed by MAD20 allelic family as it was identified. This finding revealed the pattern of distribution and suggestive of the malaria parasites strains circulating in Bayelsa state. It added to the existing knowledge of the *P.falciparum* strains predominate in Bayelsa state. Also, not understanding that *P.falciparum* with MAD20 and R033 alleles are the strains infecting subjects might be the reasons for poor management recurrent malaria in the state. This finding, pattern of allelic distribution is in agreement with patterns observed [9], [10], [23], but inconsistent with [21].

In this study, *P.falciparum* haven the msp2 stain and the FC27 and 3D7 allelic families were not detected. This might be that *P.falciparum* possessing this strain is not predominant in Bayelsa state. Although, this finding appeared to be inconsistent with previous study done [21]. The multiplicity of infection observed in this study was highest for msp1 (0.15) and lowest for msp2 (0.00) when compared with values from other works done elsewhere. Probably this might be why malaria is still very poorly treated in Bayelsa state hence, making this disease endemic in the state. This finding was contrary to other work done [10], This finding also showed that the association between the multiplicity of infection and parasitaemia levels in the *P.falciparum* infected subjects in selected hospitals in Bayelsa state is statistically significant.

## CONCLUSION

These results demonstrated high genetic diversity only in msp1 strain of *Plasmodium falciparum* with the MAD20 and R033 alleles. Therefore, it had imperative that drugs design in treating malaria in Bayelsa state should be targeted towards the msp1 strains of *Plasmodium falciparum*.

## ACKNOWLEDGEMENT

Mr Ishmael Ebidimie- (General University Hospital, Sagbama), Mrs Nake Nelson. (Federal Medical Centre Otuoke) and Mrs Martha Ukpog (Federal Medical Centre Yenagoa (They greatly assisted in sample collection)

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# ASSESSMENT OF SOME HAEMATOLOGICAL INDICES OF TUBERCULOSIS PATIENTS ACCESSING CARE IN SOME HEALTH FACILITIES IN YENAGOA, BAYELSA STATE SOUTH-SOUTH NIGERIA.

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## ABSTRACT

**T**uberculosis (TB) is a major health concern and leading infectious killer disease caused in man by the bacterium *Mycobacterium tuberculosis*. Tuberculosis as a disease is known to affect the haematological balance of patients. This study therefore was carried out to evaluate the effect of tuberculosis and its therapy on some haematological parameters in tuberculosis subjects accessing care in some health facilities in Yenagoa. The place of study was the Tuberculosis and Leprosy Hospital Igbogene, the Federal Medical Center Yenagoa (FMCY) and Niger Delta University Teaching Hospital (NDUTH) all in Bayelsa state. A Longitudinal study design was adopted to recruit 50 newly diagnosed TB subjects and 50 apparently healthy control subjects (who are age and sex matched) and monitored at two months interval until the end of their treatment of six (6) months. Sputum samples were used for AFB, whole blood for the analysis of HB, PCV, RBC, Platelet and WBC. Ziehl-Neelson and the Gene Xpert were employed to analyze the sputum samples while Abacus 380 haematology autoanalyzer was used to assay the haematological indices. The test of significance was determined by analysis of variance (ANOVA) and post hoc. Values with  $p < 0.05$  were considered significant. The result shows that PCV, Hb, and RBC were significantly lower in value ( $p < 0.05$ ), ( $38.65 \pm 4.20\%$  vs  $29.67 \pm 6.19\%$ ,  $13.13 \pm 1.08 \text{ g/dl}$  vs  $10.48 \pm 1.55 \text{ g/dl}$  and  $5.05 \pm 0.63 \times 10^{12}/\text{l}$  vs  $3.92 \pm 0.51 \times 10^{12}/\text{l}$ ) respectively, while WBC and platelets increased significantly ( $6.21 \pm 1.89 \times 10^9/\text{l}$  vs  $13.61 \pm 1.32 \times 10^9/\text{l}$ ,  $214.50 \pm 64.45 \times 10^3/\text{l}$  vs  $501.34 \pm 60.23 \times 10^3/\text{l}$ ) respectively when controls were compared to the newly diagnosed subjects. The values of all parameters monitored at two months interval of treatment are seen to be returning towards control values. Significantly the study revealed that all parameters studied reverted back to almost control values at the end of the fourth month of treatment. There were no significant difference between values as at the end of the fourth month and at the end of the sixth month of treatment ( $39.19 \pm 3.79\%$  vs  $39.32 \pm 3.03\%$  and  $p = 0.869$  for PCV,  $12.70 \pm 0.90 \text{ g/dl}$  vs  $13.05 \pm 0.90 \text{ g/dl}$ ,  $p = 0.332$  for Hb.,  $5.03 \pm 0.31 \times 10^{12}/\text{l}$  vs  $5.12 \pm 0.26 \times 10^{12}/\text{l}$ ,  $P = 0.149$  for RBC,  $6.68 \pm 1.99 \times 10^9/\text{l}$  vs  $6.48 \pm 2.93 \times 10^9/\text{l}$ ,  $p = 0.720$  for RBC and  $225.58 \pm 74.60 \times 10^3/\text{l}$  vs  $220.74 \pm 80.61 \times 10^3/\text{l}$ ,  $p = 0.781$  for platelet). We conclude by saying researchers should carry out further test to look at the possibility of ending anti-tuberculosis therapy at four (months).

**Keywords:** Tuberculosis, Longitudinal, Platelet

## INTRODUCTION

**T**uberculosis (TB) is an infectious disease that most often affects the lungs and is caused by a type of bacteria. It spreads through the air when infected people cough, sneeze or spit. Tuberculosis, a deadly and contagious infection, has killed more people than any other infectious diseases in the previous two centuries, though it has been around for hundreds of years<sup>1,2,3</sup>.

When inhaled the bacillus enters the body via droplets or dust particles containing it. The infection is categorized as pulmonary tuberculosis or non-pulmonary tuberculosis. In pulmonary tuberculosis, the pulmonary aveoli and surrounding lymph glands are lodged by the bacilli resulting in lesion characterized by acute inflammatory reactions with accumulation of fluid and white blood cells around the aveoli, while the non-pulmonary tuberculosis includes the renal and urogenital tuberculosis, miliary tuberculosis and tuberculosis meningitis with varying symptoms<sup>4</sup>.

In 2019, TB remained the most common cause of death from a single infectious pathogen. Globally, an estimated 10.0 million people developed TB disease in 2019, and there were an estimated 1.2 million TB deaths among HIV-negative people and an additional 208,000 deaths among people living with HIV. Adults accounted for 88% and children, aged <15 years, for 12% of all people with TB.

Most people who developed TB in 2019 were in the WHO regions of South-East Asia (44%), Africa (25%), and the Western Pacific (18%), with smaller percentages in the Eastern Mediterranean (8.2%), the Americas (2.9%) and

Europe (2.5%). Eight countries accounted for two thirds of the global total: India (26%), Indonesia (8.5%), China (8.4%), the Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%) and South Africa (3.6%)<sup>5</sup>. The disease burden in Nigeria is rated as the highest in Africa<sup>6</sup>.

Tuberculosis not only affects lungs but also the bone marrow and causes significant hematological abnormalities such as anemia, leukocytosis neutrophilia, lymphopenia, thrombocytopenia and also elevated ESR in the patients<sup>7</sup>.

Haematological parameters are those that are related to blood and blood forming organs; they are altered in values in most sicknesses and diseases and in chemotherapy. The most common haematological parameters are the indicators of haemoglobin concentration<sup>8</sup>. Hematological parameters like hemoglobin, Packed Cell Volume (PCV), red blood cell (RBC) count, blood indices, platelet count, white blood cell (WBC) count, erythrocyte sedimentation rate (ESR) can be used for diagnosis, prognosis, and follow-up of patients<sup>9</sup>.

In order to prevent and control this disease, early diagnosis and treatment is a necessity, and monitoring TB patients during treatment is imperative for better outcomes<sup>10</sup>.

This study was carried out to investigate the haematological profile of TB patients in the disease state as well as during and at the end of treatment which will help in the monitoring of treatment responses.

## MATERIALS AND METHOD

This was a prospective study carried out in three facilities in Bayelsa state Nigeria namely the Federal

Medical Center (FMCY), Niger Delta University Teaching Hospital (NDUTH) and the Tuberculosis and Leprosy Hospital for a duration of ten (10) months from February 2022 to November of the same year. Fifty newly diagnosed Tuberculosis and fifty apparently healthy controls subjects who were age and sex matched participated in the study. Tuberculosis subjects were monitored at two months interval of treatment until the end of the treatment period (six months).

Ethical approval was sought for and obtained from the ministry of Health Bayelsa State, Research and Ethics committee in FMCY and from a similar committee at NDUTH before the commencement of the work. Written consent was sought from all the participating subjects and only those who gave theirs were enrolled.

Sputum was collected in a sterile wide mouthed container. Three samples made up of spot, early morning and spot were collected from each patient. Two and half millilitres (2.5mls) of the blood was also collected by venous puncture and dispensed into a prepared Ethylene Diamine Tetracetic Acid (EDTA) container holding 0.05 ml of 10% of EDTA

Sputum analysis was by the Ziehl Neelson (AFB) technique as described by Ochei and Kolhatkar<sup>11</sup>, and the Genexpert technique by David Alan and Cepheid Inc. as modified by Catharina *et. al*<sup>12</sup>.

Full blood count was assayed by the method of Chabra, (2018)<sup>13</sup> using **Abacus 380 Haematology Autoanalyzer**.

Parameters: WBC, RBC, Platelet, PCV, and Hb determination. The operating principle of the Abacus 380 autoanalyzer uses the impedance method also known as the coulter-method. This method counts/sizes cells by detecting and measuring changes in electrical impedance when a particle in a conducting liquid passes through a small aperture. For the Haemoglobin measurement, the sample is lysed and diluted and is measured photometrically.

The reagent lyses the red blood cells which releases haemoglobin. The chemical process forms a stable form of methaemoglobin and this is measured by a photometer in the chamber and the results read in the displayed screen.

## RESULTS

Figure 4.1 gives some features of the subjects. It shows that the control subjects were made up of 21 males and 29 females while the newly diagnosed TB subjects also consists of 21 males and 29 females. In the 1<sup>st</sup> treatment phase, there was a decline in the number of test subjects.

The initial number of 50 was reduced to 46 (19 males and 27 females). In the 2<sup>nd</sup> treatment phase, it reveals a further decline in the test subjects from 46 in the 1<sup>st</sup> treatment phase to 41 in this phase made up of 17 males and 24 females. Lastly, the figure reveals that the 3<sup>rd</sup> treatment phase has 39 subjects made up of 16 male and 23 females.



Figure 1 Some features of the subjects

Table 1; ANOVA for Haematological variables in Newly diagnosed TB subjects, 1<sup>st</sup> treatment TB subjects, 2<sup>nd</sup> treatment TB subjects and 3<sup>rd</sup> treatment TB subjects.

| Variables<br>(Mean±SD)     | Newly<br>diagnosed TB<br>subjects<br>(n=50) | 1 <sup>st</sup> treatment TB<br>subjects<br>(n=46) | 2 <sup>nd</sup> treatment<br>TB subjects<br>(n=41) | 3 <sup>rd</sup> treatment<br>TB subjects<br>(n=39) | F-<br>value | p<br>value |
|----------------------------|---|--|--|--|-------------|------------|
| PCV (%)                    | 29.67±5.23                                  | 35.00±5.36   | 39.19± 3.79  | 39.32± 3.03  | 45.687      | 0.000      |
| Hb (g/dl)                  | 10.48±1.55                                  | 11.66±1.58   | 12.70±0.90   | 13.05±0.90   | 56.324      | 0.000      |
| RBC(x10 <sup>12</sup> /l)  | 3.92±0.51                                   | 4.45 ± 0.51  | 5.03± 0.31   | 5.12± 0.26   | 77.07       | 0.000      |
| WBC(x10 <sup>9</sup> /l)   | 13.61±1.32                                  | 8.98 ± 3.02  | 6.68±1.99  | 6.48±2.93  | 88.79       | 0.000      |
| Plt (x10 <sup>12</sup> /l) | 501.34±60.23                                | 391.86±153.28                                      | 225.58±74.60                                       | 220.74±80.61                                       | 84.40       | 0.000      |

subjects.

**KEY**  
WBC; White Blood Count; RBC; Red Blood Count; HB; Haemoglobin estimation;  
PCV; Packed Cell Volume; PLT; Platelet

Table 2a; Post-Hoc for Haematological variables in Controls, Newly diagnosed TB subjects and 1<sup>st</sup> treatment TB subjects.

| Parameter                  |         | Group 1      | Group 2      | p-value | Group 1      | Group 3      | p-value |
|----------------------------|---------|--------------|--------------|---------|--------------|--------------|---------|
|                            | N       | 50           | 50           |         | 50           | 46           |         |
| PCV (%)                    | Mean±SD | 38.65±4.20   | 29.67±6.19   | 0.000   | 38.65±4.20   | 35.00±5.36   | 0.000   |
| Hb (g/dl)                  | Mean±SD | 13.13±1.08   | 10.48±1.55   | 0.000   | 13.33±1.08   | 11.66 ±1.58  | 0.000   |
| RBC(x10 <sup>12</sup> /l)  | Mean±SD | 5.05±0.63    | 3.92±0.51    | 0.000   | 5.05±0.63    | 4.45 ± 0.51  | 0.000   |
| WBC(x10 <sup>9</sup> /l)   | Mean±SD | 6.21±1.89    | 13.61±1.32   | 0.000   | 6.21±1.89    | 8.98 ± 3.02  | 0.013   |
| Plt (x10 <sup>12</sup> /l) | Mean±SD | 214.50±64.45 | 501.34±60.23 | 0.000   | 214.50±64.45 | 391.86±153.2 | 0.000   |

WBC; White Blood Count  
RBC; Red Blood Count  
HB; Haemoglobin estimation  
PCV; Packed Cell Volume  
PLT; Platelet

Group 1, Control subjects  
Group 2; Newly diagnosed TB subjects  
Group 3; 1<sup>st</sup> Treatment TB subject  
Group 4; 2<sup>nd</sup> Treatment TB subjects  
Group 5; 3<sup>rd</sup> Treatment TB subjects



**Table 2b; Post-Hoc for Haematological variables in Controls, 2<sup>nd</sup> treatment TB subjects and 3<sup>rd</sup> treatment TB subjects.**

| Parameter                  |         | Group 1      | Group 4      | p-value | Group 1      | Group 5      | p-value |
|----------------------------|---------|--------------|--------------|---------|--------------|--------------|---------|
|                            | N       | 50           | 41           |         | 50           | 39           |         |
| PCV (%)                    | Mean±SD | 38.65±4.20   | 39.19± 3.79  | 0.526   | 38.65±4.20   | 39.32± 3.03  | 0.729   |
| Hb (g/dl)                  | Mean±SD | 13.13±1.08   | 12.70±0.90   | 0.187   | 13.13±1.08   | 13.05±0.90   | 0.187   |
| RBC(x10 <sup>12</sup> /l)  | Mean±SD | 5.05±0.63    | 5.03± 0.31   | 0.868   | 5.05±0.63    | 5.12± 0.26   | 0.484   |
| WBC(x10 <sup>9</sup> /l)   | Mean±SD | 6.21±1.89    | 6.68±1.99    | 0.253   | 6.21±1.89    | 6.48±2.93    | 0.603   |
| Plt (x10 <sup>12</sup> /l) | Mean±SD | 214.50±64.45 | 225.58±74.60 | 0.449   | 214.50±64.45 | 220.74±80.61 | 0.686   |

WBC; White Blood Count  
RBC; Red Blood Count  
HB; Haemoglobin estimation  
PCV; Packed Cell Volume  
PLT; Platelet

Group 1; Control subjects  
Group 2; Newly diagnosed TB subjects  
Group 3; 1<sup>st</sup> Treatment TB subject  
Group 4; 2<sup>nd</sup> Treatment TB subjects  
Group 5; 3<sup>rd</sup> Treatment TB subjects

**Table 2c; Post-Hoc for Haematological variables in Newly diagnosed TB subjects, 1<sup>st</sup> treatment TB subjects and 2<sup>nd</sup> treatment TB subjects**

| Parameter                  |         | Group 2     | Group 3     | p-value | Group 2    | Group 4     | p-value |
|----------------------------|---------|-------------|-------------|---------|------------|-------------|---------|
|                            | N       | 50          | 46          |         | 50         | 41          |         |
| PCV (%)                    | Mean±SD | 29.67±6.19  | 35.00±5.36  | 0.000   | 29.67±6.19 | 39.19± 3.79 | 0.000   |
| Hb (g/dl)                  | Mean±SD | 10.48±1.55  | 11.66 ±1.58 | 0.000   | 10.48±1.55 | 12.70±0.90  | 0.000   |
| RBC(x10 <sup>12</sup> /l)  | Mean±SD | 3.92±0.51   | 4.45 ± 0.51 | 0.000   | 3.92±0.51  | 5.03± 0.31  | 0.000   |
| WBC(x10 <sup>9</sup> /l)   | Mean±SD | 13.61±1.32  | 8.98 ± 3.02 | 0.000   | 13.61±1.32 | 6.68±1.99   | 0.000   |
| Plt (x10 <sup>12</sup> /l) | Mean±SD | 501.34±60.3 | 391.86±153  | 0.000   | 501.34±60  | 225.58±74.6 | 0.000   |

WBC; White Blood Count  
RBC; Red Blood Count  
HB; Haemoglobin estimation  
PCV; Packed Cell Volume  
PLT; Platelet

Group 1; Control subjects  
Group 2; Newly diagnosed TB subjects  
Group 3; 1<sup>st</sup> Treatment TB subject  
Group 4; 2<sup>nd</sup> Treatment TB subjects  
Group 5; 3<sup>rd</sup> Treatment TB subjects

Table 2d; Post-Hoc for Haematological variables in Newly diagnosed TB subjects, 1<sup>st</sup> treatment TB subjects, 2<sup>nd</sup> treatment TB subjects and 3<sup>rd</sup> treatment TB subjects.

| parameter                  |         | Group 2    | Group 5      | p-value | Group 3     | Group 4      | p-value |
|----------------------------|---------|------------|--------------|---------|-------------|--------------|---------|
|                            | N       | 50         | 39           |         | 46          | 41           |         |
| PCV (%)                    | Mean±SD | 29.67±6.19 | 39.32± 3.03  | 0.000   | 35.00±5.36  | 39.19± 3.79  | 0.000   |
| Hb (g/dl)                  | Mean±SD | 10.48±1.55 | 13.05±0.90   | 0.000   | 11.66 ±1.58 | 12.70±0.90   | 0.000   |
| RBC(x10 <sup>12</sup> /l)  | Mean±SD | 3.92±0.51  | 5.12± 0.26   | 0.000   | 4.45 ± 0.51 | 5.03± 0.31   | 0.000   |
| WBC(x10 <sup>9</sup> /l)   | Mean±SD | 13.61±1.32 | 6.48±2.93    | 0.000   | 8.98 ± 3.02 | 6.68±1.99    | 0.000   |
| Plt (x10 <sup>12</sup> /l) | Mean±SD | 501.34±60. | 220.74±80.61 | 0.000   | 391.86±1532 | 225.58±74.60 | 0.000   |

WBC; White Blood Count  
RBC; Red Blood Count  
HB; Haemoglobin estimation  
PCV; Packed Cell Volume  
PLT; Platelet

Group 1, Control subjects  
Group 2; Newly diagnosed TB subjects  
Group 3; 1<sup>st</sup> Treatment TB subject  
Group 4; 2<sup>nd</sup> TreatmentTB subjects  
Group 5; 3<sup>rd</sup> Treatment TB subjects

Table 2e; Post-Hoc for Haematological variables in Newly diagnosed TB subjects, 1<sup>st</sup> treatment TB subjects, 2<sup>nd</sup> treatment TB subjects and 3<sup>rd</sup> treatment TB subjects.

| Parameter                  |         | Group 3      | Group 5      | p-value | Group 4      | Group 5      | p-value |
|----------------------------|---------|--------------|--------------|---------|--------------|--------------|---------|
|                            | N       | 46           | 39           |         | 41           | 39           |         |
| PCV (%)                    | Mean±SD | 35.00±5.36   | 39.32± 3.03  | 0.000   | 39.19± 3.79  | 39.32± 3.03  | 0.869   |
| Hb (g/dl)                  | Mean±SD | 11.66 ±1.58  | 13.05±0.90   | 0.000   | 12.70±0.90   | 13.05±0.90   | 0.332   |
| RBC(x10 <sup>12</sup> /l)  | Mean±SD | 4.45 ± 0.51  | 5.12± 0.26   | 0.000   | 5.03± 0.31   | 5.12± 0.26   | 0.149   |
| WBC(x10 <sup>9</sup> /l)   | Mean±SD | 8.98 ± 3.02  | 6.48±2.93    | 0.000   | 6.68±1.99    | 6.48±2.93    | 0.720   |
| Plt (x10 <sup>12</sup> /l) | Mean±SD | 391.86±153.2 | 220.74±80.61 | 0.000   | 225.58±74.60 | 220.74±80.61 | 0.781   |

WBC; White Blood Count  
RBC; Red Blood Count  
HB; Haemoglobin estimation  
PCV; Packed Cell Volume  
PLT; Platelet

Group 1, Control subjects  
Group 2; Newly diagnosed TB subjects  
Group 3; 1<sup>st</sup> Treatment TB subject  
Group 4; 2<sup>nd</sup> TreatmentTB subjects  
Group 5; 3<sup>rd</sup> Treatment TB subjects

Table 1. is the ANOVA for Haematological variables in Newly diagnosed

TB subjects, 1<sup>st</sup> treatment TB subjects, 2<sup>nd</sup> treatment TB subjects and 3<sup>rd</sup> treatment TB subjects. There were significant increases ( $p = 0.000$ ) in the values of PCV, Hb and RBC when comparison were made from Newly diagnosed TB subjects to 3<sup>rd</sup> treatment TB subjects. Similarly, there were significant decreases ( $p = 0.000$ ) in the values of WBC and Plt when the mean values of Newly diagnosed TB subjects to 3<sup>rd</sup> treatment TB subjects were compared.

Table 2a is the Post-Hoc analysis for Haematological variables in Controls, Newly diagnosed TB subjects and 1<sup>st</sup> treatment TB subjects. There were significantly lower Mean $\pm$ SD values of PCV, Hb and RBC ( $p = 0.000$ ,  $p = 0.000$  and  $p = 0.000$  respectively) in Newly diagnosed TB subjects when compared to controls. The control and Newly diagnosed values respectively are  $38.65 \pm 4.20 / 29.67 \pm 6.19$  % for PCV,  $13.13 \pm 1.08 / 10.48 \pm 1.55$  g/dl for Hb and  $5.05 \pm 0.63 / 3.92 \pm 0.51 \times 10^{12}/l$  for RBC. The Mean $\pm$ SD values of WBC and Plt were significantly higher in Newly diagnosed TB subjects ( $p = 0.000$  and  $p = 0.000$  respectively) when compared to controls with values of  $6.21 \pm 1.89 / 13.61 \pm 1.32 \times 10^9/l$  and  $214.50 \pm 64.45 / 501.34 \pm 60.23 \times 10^{12}/l$ .

There were significantly lower Mean $\pm$ SD values of PCV, Hb and RBC ( $p = 0.000$ ,  $p = 0.000$  and  $p = 0.000$  respectively) in 1<sup>st</sup> treatment TB subjects when compared to controls. The control and 1<sup>st</sup> treatment values respectively are  $38.65 \pm 4.20 / 35.00 \pm 5.36$  % for PCV,  $13.33 \pm 1.08 / 11.66 \pm 1.58$  g/dl for Hb and  $5.05 \pm 0.63 / 4.45 \pm 0.51 \times 10^{12}/l$  for RBC.

The Mean $\pm$ SD values of WBC and Plt were significantly higher in 1<sup>st</sup> treatment TB subjects ( $p = 0.013$  and  $p = 0.000$  respectively) when compared to controls with values of  $6.21 \pm 1.89 / 8.98 \pm 3.02 \times 10^9/l$  and  $214.50 \pm 64.45 / 391.86 \pm 153.28 \times 10^{12}/l$ .

Table 2b is the Post-Hoc for Haematological variables in Controls, 2<sup>nd</sup> treatment TB subjects and 3<sup>rd</sup> treatment TB subjects.

There was no significant difference ( $p = 0.526$ ) in the value of PCV,  $38.65 \pm 4.20$  % in controls compared to  $39.19 \pm 3.79$  % in 2<sup>nd</sup> treatment TB subjects. Similarly there was no significant difference ( $p = 0.187$ ) in the value of Hb,  $13.33 \pm 1.08$  g/dl in controls compared to  $12.70 \pm 0.90$  g/dl in 2<sup>nd</sup> treatment TB subjects. All the rest parameters in the table followed the same pattern. There were no significant difference in the value of RBC ( $p = 0.868$ ), WBC ( $p = 0.253$ ) and Plt ( $p = 0.449$ ) when control values  $5.05 \pm 0.63 \times 10^{12}/l$ ,  $6.21 \pm 1.89 \times 10^9/l$  and  $214.50 \pm 64.45 \times 10^{12}/l$  was compared to 2<sup>nd</sup> treatment TB values of  $5.03 \pm 0.31 \times 10^{12}/l$ ,  $6.68 \pm 1.99 \times 10^9/l$  and  $225.58 \pm 74.60 \times 10^{12}/l$  respectively.

There was no significant difference in the value of PCV,  $38.65 \pm 4.20$  % in controls compared to  $39.32 \pm 3.03$  % in 3<sup>rd</sup> treatment TB subjects ( $P = 0.729$ ). The value of Hb,  $13.33 \pm 1.08$  g/dl in controls was not significant when compared with  $13.05 \pm 0.90$  g/dl in 3<sup>rd</sup> treatment TB subjects. The table also reveals that there were no significant differences in the value of RBC ( $p = 0.484$ ), WBC ( $p = 0.603$ ) and Plt ( $p = 0.686$ ) when control values  $5.05 \pm 0.63 \times 10^{12}/l$ ,  $6.21 \pm 1.89 \times 10^9/l$  and  $214.50 \pm 64.45 \times 10^{12}/l$  was compared to 3<sup>rd</sup> treatment TB values of  $5.12 \pm 0.26 \times 10^{12}/l$ ,  $6.48 \pm 2.93 \times 10^9/l$  and  $220.74 \pm 74.60 \times 10^{12}/l$  respectively.

Table 2c is the Post-Hoc for Haematological variables in Newly diagnosed TB subjects, 1<sup>st</sup> treatment TB subjects 2<sup>nd</sup> treatment TB subjects. There were significantly increased ( $p = 0.000$ ) values in the mean levels of PCV, Hb and RBC  $35.00 \pm 5.36$  %,  $11.66 \pm 1.58$  g/dl and  $4.45 \pm 0.51 \times 10^{12}/l$  in 1<sup>st</sup> treatment TB subjects compared to values of Newly diagnosed TB subject  $29.67 \pm 5.23$  %,  $10.48 \pm 1.55$  g/dl and  $3.92 \pm 0.51 \times 10^{12}/l$  respectively. The mean values of WBC and Plt were significantly decreased ( $P = 0.000$ ) when 1<sup>st</sup> treatment TB subjects ( $8.98 \pm 3.02 \times 10^9/l$  and  $391.86 \pm$

$153.28 \times 10^{12}/l$ ) was compared to newly diagnosed TB subjects ( $13.61 \pm 1.32 \times 10^9/l$  and  $501.34 \pm 60.23 \times 10^{12}/l$ ).

There were significantly increased ( $p = 0.000$ ) values in the mean levels of PCV, Hb and RBC  $39.19 \pm 3.79$  %,  $12.70 \pm 0.90$  g/dl and  $5.03 \pm 0.31 \times 10^{12}/l$  in 2<sup>nd</sup> treatment TB subjects compared to values of Newly diagnosed TB subject  $29.67 \pm 5.23$  %,  $10.48 \pm 1.55$  g/dl and  $3.92 \pm 0.51 \times 10^{12}/l$  respectively. The mean values of WBC and Plt were significantly decreased ( $P = 0.000$ ) when 2<sup>nd</sup> treatment TB subjects ( $6.68 \pm 1.99 \times 10^9/l$  and  $391.86 \pm 225.58 \pm 74.60 \times 10^{12}/l$ ) was compared to newly diagnosed TB subjects ( $13.61 \pm 1.32 \times 10^9/l$  and  $501.34 \pm 60.23 \times 10^{12}/l$ ).

Table 2d is the Post-Hoc for Haematological variables in Newly diagnosed TB subjects, 1<sup>st</sup> treatment TB subjects, 2<sup>nd</sup> treatment TB subjects and 3<sup>rd</sup> treatment TB subjects.

There were significantly increased mean levels of PCV ( $P = 0.000$ ), Hb ( $p = 0.000$ ) and RBC ( $p = 0.000$ ) when mean values of Newly diagnosed TB subjects  $29.67 \pm 5.23$  %,  $10.48 \pm 1.55$  g/dl and  $3.92 \pm 0.51 \times 10^{12}/l$  were compared to that of 3<sup>rd</sup> treatment TB subjects  $39.32 \pm 3.03$  %,  $13.05 \pm 0.90$  g/dl and  $5.12 \pm 0.26 \times 10^{12}/l$  respectively. There were significantly reduced mean levels of WBC ( $p = 0.000$ ) and Plt ( $p = 0.000$ ) when mean values of Newly diagnosed TB subjects  $13.61 \pm 1.32 \times 10^9/l$  and  $501.34 \pm 60.23 \times 10^{12}/l$  were compared to that of 3<sup>rd</sup> treatment TB subjects  $6.48 \pm 2.93 \times 10^9/l$  and  $220.74 \pm 80.61 \times 10^{12}/l$  respectively.

There were significantly increased mean levels of PCV ( $P = 0.000$ ), Hb ( $p = 0.000$ ) and RBC ( $p = 0.000$ ) when mean values of 1<sup>st</sup> treatment TB subjects  $35.00 \pm 5.36$  %,  $11.66 \pm 1.58$  g/dl and  $4.45 \pm 0.51 \times 10^{12}/l$  were compared to that of 2<sup>nd</sup> treatment TB subjects  $39.19 \pm 3.79$  %,  $12.70 \pm 0.90$  g/dl and  $5.03 \pm 0.31 \times 10^{12}/l$  respectively. There were significantly reduced mean levels of WBC ( $p = 0.000$ ) and Plt ( $p = 0.000$ ) when mean values of 1<sup>st</sup> treatment TB subjects  $8.98 \pm 3.02 \times 10^9/l$  and  $391.86 \pm 153.28 \times 10^{12}/l$  were compared to that of 2<sup>nd</sup> treatment TB subjects  $6.68 \pm 1.99 \times 10^9/l$  and  $225.58 \pm 74.60 \times 10^{12}/l$  respectively.

Table 2e is the Post-Hoc for Haematological variables in 1<sup>st</sup> treatment TB subjects, 2<sup>nd</sup> treatment TB subjects and 3<sup>rd</sup> treatment TB subjects. There were significantly increased mean levels of PCV ( $P = 0.000$ ), Hb ( $p = 0.000$ ) and RBC ( $p = 0.000$ ) when mean values of 1<sup>st</sup> treatment TB subjects  $35.00 \pm 5.36$  %,  $11.66 \pm 1.58$  g/dl and  $4.45 \pm 0.51 \times 10^{12}/l$  were compared to that of 3<sup>rd</sup> treatment TB subjects  $39.32 \pm 3.03$  %,  $13.05 \pm 0.90$  g/dl and  $5.12 \pm 0.26 \times 10^{12}/l$  respectively.

There were significantly reduced mean levels of WBC ( $p = 0.000$ ) and Plt ( $p = 0.000$ ) when mean values of 1<sup>st</sup> treatment TB subjects  $8.98 \pm 3.02 \times 10^9/l$  and  $391.86 \pm 153.28 \times 10^{12}/l$  were compared to that of 3<sup>rd</sup> treatment TB subjects  $6.48 \pm 2.93 \times 10^9/l$  and  $220.74 \pm 80.61 \times 10^{12}/l$  respectively.

There was no significant difference ( $P = 0.869$ ) in the value of PCV,  $39.19 \pm 3.79$  % in 2<sup>nd</sup> treatment TB subjects compared to  $39.32 \pm 3.03$  % in 3<sup>rd</sup> treatment TB subjects. The value of Hb,  $12.70 \pm 0.90$  g/dl in 2<sup>nd</sup> treatment TB subjects was not significant when compared with  $13.05 \pm 0.90$  g/dl in 3<sup>rd</sup> treatment TB subjects ( $p = 0.332$ ). The table also reveals that there were no significant differences in the value of RBC ( $p = 0.149$ ), WBC ( $p = 0.720$ ) and Plt ( $p = 0.781$ ) when 2<sup>nd</sup> treatment TB subjects values  $5.03 \pm 0.31 \times 10^{12}/l$ ,  $6.68 \pm 1.99 \times 10^9/l$  and  $225.58 \pm 74.60 \times 10^{12}/l$  were compared to 3<sup>rd</sup> treatment TB values of  $5.12 \pm 0.26 \times 10^{12}/l$ ,  $6.48 \pm 2.93 \times 10^9/l$  and  $220.74 \pm 74.60 \times 10^{12}/l$  respectively.

## Discussion

In this study, the effects of tuberculosis were clearly demonstrated on the haematological parameters. One of the major findings in this study was that tuberculosis infection adversely affected haematological parameters either as an increase or decrease in value. There were lower values of

Hb, PCV and RBC. In the first analysis where the means of the control subjects and newly diagnosed TB subjects were compared, there was a statistically significance ( $P < 0.05$ ) decrease observed on these parameters. The reason for reduced haemoglobin level in tuberculosis disease has been attributed to poor nutritional status of the patients and poor absorption of micro-nutrients needed for blood production. Pulmonary tuberculosis being a chronic ailment, impacts negatively on the haemopoietic system leading to a decrease in erythropoiesis.<sup>14,15</sup> However, the Hb and PCV values among the newly diagnosed TB subjects were observed to be high as compared to other studies such as the work of Akpan *et al.*, 2012<sup>15</sup> and Nwankwo *et al.*, 2005<sup>16</sup> in Calabar and Kano respectively. This could be attributed to the culture of high intake of haematinics by sick persons and perhaps the native diets in the area rich in iron such as periwinkles, fish and other sea foods.

The haematological parameters that increased in value were the platelets and WBC. The mean  $\pm$  SD for platelet when compared with the control, shows a statistically significant increase with a p value of  $P = 0.000$  applying the students T-test analysis. This rise in platelet number might be related to increased levels of interleukin 6 and may lead to a hypercoagulable state and deep vein thrombosis<sup>17</sup>. The same theory was postulated by Lieberth *et al.*, 2015<sup>18</sup>. Our findings are in agreement with the findings of Andrew and Edwin, 2013 who reported that *Mycobacterium tuberculosis* infection is associated with thrombocytosis<sup>17</sup>.

The WBC for the control and test group had a P value of  $P = 0.000$ . In bacteriological infection, there is always the response from the body's immune system resulting to an elevated count of white cells above normal (leucocytosis). This was also the finding of Rohini *et al.*, 2016<sup>19</sup>, Wang *et al.*, 2021<sup>20</sup> and Barreda *et al.*, 2020<sup>21</sup> who reported elevated counts of WBC in tuberculosis infection.

The second part of this discussion focuses on the values of parameters under consideration in the follow-up cases during tuberculosis treatment. The tables above (Tables 2a–2f) considered the values of haematological parameters during the end of the first phase (two months) of anti TB therapy here known as the 1<sup>st</sup> treatment group and the end of the fourth (4th) month period of treatment here known as 2<sup>nd</sup> treatment group as well as the end of the 6<sup>th</sup> month of treatment here known as the 3<sup>rd</sup> treatment group.

There were significantly lower values ( $P < 0.05$ ) of PCV, Hb and RBC when 1st treatment group were compared with controls. On the contrary, significantly higher values ( $p < 0.05$ ) were seen for WBC and Plt. When Newly diagnosed group were compared with 1<sup>st</sup> treatment group, the values of PCV, Hb and RBC were significantly increased ( $p = 0.000$ ).

That of WBC and Plts were significantly ( $p < 0.05$ ) decreased. These haematological parameters under consideration were all altered significantly as at the Newly diagnosed stage but at the end of the 1<sup>st</sup> and 2<sup>nd</sup> treatment stage are seen to be returning towards control values. This suggests that all factors responsible for the alterations of these values were not permanently altered and were being reversed during the treatment. In a similar study Leonardo *et al.*, (2019)<sup>22</sup> opined that pulmonary tuberculosis (PTB) is associated with chronic inflammation and anaemia. They however could not ascertain how anaemia impacts systemic inflammation in PTB patients undergoing antituberculosis therapy (ATT). Their findings revealed that most of anaemia cases were likely caused by chronic inflammation. A distinct biosignature related to anemia was detected which is defined by increased values of uric acid, C-reactive protein, and erythrocyte sedimentation rate. Importantly, anaemic patients sustained increased levels of several biochemical markers at day 60 of therapy. They concluded their work by saying TB patients with anaemia exhibit a distinct

inflammatory profile, which is only partially reverted at day 60 of ATT<sup>22</sup>.

Furthermore, Manjunath, Sanju and Sagar (2011)<sup>23</sup>, reported that Autoimmune haemolytic anemia (AIHA) represents a spectrum of disorders in which antibodies against self antigens on the erythrocyte membrane cause a shortened red blood cell (RBC) life span. AIHA can occur as an idiopathic (primary) disorder or can coexist with another disease (secondary). AIHA can also occur following administration of certain drugs (drug induced). All these did not happen in anti TB therapy. Also they found out that Tuberculosis presents with a wide variety of haematological manifestations. Anemia in tuberculosis is most often due to nutritional deficiency, malabsorption syndromes, failure of iron utilization, and bone marrow suppression. AIHA is exceedingly rare in tuberculosis. The most common form of anaemias in TB is normocytic normochromic anemia<sup>24</sup>.

Eyuel *et al* (2016)<sup>25</sup> in their research on TB subjects in Ethiopia observed similar findings on RBCs, WBCs, Hb, PCV and Plt, and so does Sharma, Mohan & Sharma, (2012)<sup>26</sup>, who worked on the challenges on diagnosis and treatment of TB. Other similar works with the same findings are (Lugito *et al.*, 2019<sup>27</sup>, Sanchez-Codez *et al.*, 2020<sup>28</sup>, Alende-Castro *et al.*, 2020<sup>29</sup>, Elziny, 2021 and Zunt, 2018<sup>30</sup>).

Further more, it could be observed from table 2e that the comparison of the mean values between 2<sup>nd</sup> treatment group and 3<sup>rd</sup> treatment groups of all parameters studied were not significant ( $p > 0.05$ ). The inference here is that at the end of the fourth month of treatment haematological parameters were fully restored as there were also no significant difference ( $p > 0.05$ ) when there values were compared to controls.

## CONCLUSION

From the study above we want to suggest that researchers should look into the issue of six month treatment of anti- tuberculosis therapy since studied parameters were found to return to normal at the end of the fourth month of treatment.

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