# PREVALENCE OF URINARY SCHISTOSOMIASIS AND ITS CO-INFECTION WITH SALMONELLA SPECIES AMONG PUPILS IN JABA LOCAL GOVERNMENT AREA, KADUNA STATE, NIGERIA

**BY**

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**OCTOBER, 2016**

## DECLARATION

I declare that the work in this dissertation entitled "**Prevalence of urinary schistosomiasis and its co-infection with *Salmonella* species among pupils in JabaLocal Government Area, Kaduna State, Nigeria**"has been carried out by me in the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria. The pieces of information derived from the literature have been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

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|---|---|---|
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## CERTIFICATION

This dissertation entitled "**Prevalence of urinary schistosomiasis and its co-infection with *Salmonella* species among pupils in JabaLocal Government Area, Kaduna State, Nigeria**"by Gabriel Bishop **Henry**meets the regulation governing the award of Masters of Science (M.Sc.) Microbiology degree of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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## DEDICATION

This work is dedicated to God and everyone that has contributed to itsreality.

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## ABBREVIATIONS, DEFINITIONS, GLOSSARY

| | |
|---|---|
| % | Percentage (or proportion) of |
| 'Fadama' | Marshy/waterlogged land for cultivation of rice, cocoyam, sugarcane |
| ANOVA | Analysis of Variation |
| BSA | Bismuth Sulphite Agar |
| CDC | Centers for Disease Prevention and Control |
| CLED | Cystine-lactose-electrolyte deficient agar |
| DNA | Deoxyribonucleic acid |
| E, N | East, North latitudes |
| EDTA | Ethylene-diamine-tetraacetic acid |
| *et al.* | And other authors |
| FGS | Female genital schistosomiasis |
| $H_0$ | Null hypothesis |
| $H_2S$ | Hydrogen sulphide |
| HCT | microhaematocrit centrifuge technique |
| HE | Hektoen Enteric agar |
| HIV/AIDS | Human immunodeficiency virus/Acquired immunodeficiency syndrome |
| hr./min. | Hour(s)/minute(s): units of time |
| KDSUBED | Kaduna State Universal Basic Education Board |
| KOH | Potassium hydroxide |
| LDC | Lysine decarboxylation |
| LGA | Local Government Area |
| M.Sc. | Master degree |
| MAC | MacConkey agar |
| MIL | Motility Indole Lysine medium |
| MIO | Motility Indole Ornithine medium |
| ml | milliliter: unit for smaller volume of a liquid |
| MR, VP | Methyl red test, VogesProskauer |
| $x^2$` | Chi square |
| n.d | No date of publication |
| NA | Nutrient agar |
| No. | Number |
| NTS | Nontyphoidal*Salmonella* serotypes |

| | |
|---|---|
| $^{o}$C | Degree Celsius: unit of temperature measurement |
| ODC | Ornithine decarboxylation |
| *P* | *P*-value |
| PCV | Packed cell volume |
| PHLN | Public Health Laboratory Network case definitions |
| RCF | Relative centrifugation force: force for spinning with a centrifuge |
| SSA | *Salmonella-Shigella* Agar: A selective medium for *Salmonella*, *Shigella* |
| sp, spp | species |
| Th2 | T-helper type 2 |
| TSI | Triple Sugar Iron agar |
| UK SMI | United Kingdom Standards for Microbiology Investigations |
| WHO | World Health Organization |
| XLD | Xylose Lysine Deoxycholate Agar: A selective medium for *Salmonella* |
| rpm | Revolutions per minute |

## ABSTRACT

Urinary schistosomiasis and *Salmonella* bacteraemia are increasing health burdens among African children. This study was aimed at assessing the prevalence of urinary schistosomiasisand its co-infection with *Salmonella* species amongst pupils in Jaba LGA, Kaduna State, Nigeria. A total of 505 pupils voluntarily participated in the study.Ten (10) ml urine and 2ml blood samples were collected from each pupil. The urine samples were examined microscopically for *Schistosoma haematobium* egg(s) while count/10ml of urine was recorded. Packed cell volume was determined. Blood and urine samples were pre-enriched in Selenite-F broth and Brilliant Green Bile broth and culturedon *Salmonella Shigella*Agar and Xylose-Lysine-Desoxycholate.Results were subjected statisticalanalyses at P=0.05.Overall prevalence of urinary schistosomiasis was 12.3%. There was absence of*Salmonella*species from all the samples, hence no co-infection of *Schistosoma haematobium* and *Salmonella* spp. All the blood cultures had no bacterial growth but seven different Gram-negative bacteria were isolated from the urine samples. Urinary schistosomiasis was most prevalent in villages: Bitaro (23.2%),Ankun (22.2%) and Kwoi (20.3%). There was higher occurrence of urinary schistosomiasis among the female (15.5%) than in the male pupils (9.1%).Mean intensity in the female pupils (4.18±1.202eggs/10ml) was significantly higher ($P \leq 0.05$) compared to 1.22±0.500eggs/10ml in the male. Pupils within 4-6 years were not infected,but those within 13-15 years had the highest prevalence of 27.9% withmean intensity of 9.97±3.915eggs/10ml of urine. Prevalence of anaemia was 8.1%. Painful micturition, red-colouredurine, 'Fadama' farming and anaemia were statistically associated with urinary schistosomiasis($P \leq 0.05$, OR >1).There was no awareness of schistosomiasisamong the study population. From this study, the female pupils weremore infected with urinary schistosomiasis in Jaba LGA of Kaduna State, Nigeria

# CHAPTER ONE

## 1.0 INTRODUCTION

Schistosomiasis is a chronic parasiticdisease caused by blood flukes (Osada and Kanazawa, 2011; WHO, 2016). These blood flukes (or trematode worms) are prime examples of complex multicellular pathogens that flourish in human hosts despite the presence of immune responses mounted against them (Edward and Andrew, 2002). Adult schistosomes absorb host's proteins to coat their integuments (Leder and Weller, 2011) which enable them to partially overcomehuman host's defenses andpossibly live and reproduce for many years in the host who becomes relatively resistant to new infections (Cox, 2004).

The three main species of *Schistosoma* that cause diseases in man are: *Schistosoma mansoni*(*S. mansoni*), *Schistosoma haematobium* (*S. haematobium*) and *Schistosoma japonicum*(*S.japonicum*) (Rollinson and Southgate, 1987; Jamjoom, 2006). Other distributions of*Schistosoma intercalatum*(*S. intercalatum*) in Central Africa and *Schistosoma mekongi* (*S. mekongi*) in Cambodia and Lao can also cause human infections (WHO, 2015; Clerinx and Soentjens, 2015). These worms have complex and indirect life cycles involving intermediate (snail) hosts.

Schistosomiasis is a chronic debilitating disease that affects the populations of tropical and subtropical countries, especially children who indulge in water-based activities in unsafe or cercarial-infested water bodies (Noble and Glem, 1982; Ibironke *et al.*, 2012; Kanwai *etal.*, 2011; **Elele and Ewurum, 2013; WHO, 2016)**. Among all the species of schistosomes, *S. haematobium*is the only cause of urinary schistosomiasis; all others cause intestinal schistosomiasis (Jamjoom, 2006; Ibironke *et al.*, 2012).

Urinary schistosomiasis is diagnosed by microscopic detection of *S. haematobium* eggs in a urine sample as a gold standard (Ibironke *et al.*, 2012; Barsoum, 2013). Humans become infected bypenetration (or dermo-invasion) of intact skin by cercariaewhich are attracted to the warmth of the body and skin lipids (Sakanari and Mckerrow, 2010).

Salmonellae are motile facultative anaerobes, non-spore-forming,Gram-negative bacilli, measuring 0.7 to 1.5 µmby 2 to 5 µm in size (Bell and Kyriakide, 2002; Global Salm-Surv, 2003; Molbak *et al.*, 2006; Pegues and Miller, 2009). The genus *Salmonella* belongs to the family Enterobacteriaceae (Guthrie, 1991; Meneses, 2010) consisting of two species: *Salmonella bongori* and *Salmonella enterica*(*S. enterica*) which is divided into 6 subspecies (Global Salm-Surv, 2003; Paccagnella, 2005).*Salmonella* are found in both group II and III of WHO classification of infective microorganisms (Cheesbrough, 2009). They have been identified as one of the leading causes of foodborne illnesses in the world (Cardinale*et al.*, 2005; Henry *et al.*, 2015). *Salmonella* has caused a number of outbreaks in developed and developing countries which emphasizes its importance and impact (Meneses, 2010) inadequate disposal of sewage, flooding and the consumption of unsafe water (Mohager *et al.*, 2014).

Serotyping is a phenotypic bacterial typing system, and very useful in understanding the epidemiology of *Salmonella* infections. Hence, itcan be used in tracing sources of contamination during outbreaks (Meneses, 2010). The Kauffmann-White method is a worldwide ‘gold standard’ for identification of Ohne (O) and Hauch (H) antigens of *Salmonella* serotypes (Pegues and Miller, 2009). It provides information on the severity of the diseases, the source of contamination and their resistance patterns (Molbak *et al.*, 2006), as well as surveillance data collection over a period of time (Oslen *et al.*, 2001). As at the year 2000, about 2,449 known serotypes of *Salmonella* wereреported (Brenner *et al.*, 2000) but hadincreased to 2,541 serotypes (Pegues and Miller, 2009).Complete

nomenclature for *Salmonella* is complex and appears thus: *Salmonella enterica* subspecies*enterica* serovarTyphimurium (Brenner *et al.*, 2000; Molbak, 2006), but it can simply be written as *S.* Typhimurium (Cheesbrough, 2009). The genus name,whether in full or abbreviated, is italicized but the serovar (also called serotype) is not.

Typhoid and paratyphoid fevers are commonly grouped as enteric fever (White, 2010)and are common in the less-industrialized countries (Mohager *et al.*, 2014). Typhoid is caused by *Salmonella* Typhi (Threlfall *et al.*, 2010) butParatyphoid can either be caused by *Salmonella* Paratyphi A, *Salmonella* Paratyphi B or *Salmonella* Paratyphi C. Children tend to have higher bacteraemia than adults (White, 2010). Usage of ineffective antibiotics has contributed to emergence of antibiotic resistance as well as the phenomenon of bacterial persistence (Barnhill *et al.*, 2011). Infection caused by *Salmonella* Typhi (*S.* Typhi)is a major public health problem in most developing countries.There areincreasing deaths dueto typhoid fever especially in areas with emergence of *S.* Typhi resistant to previously administered antibiotics. In the endemic areas, typhoid fever occurs most frequently among children and young adults of 3-19 years old (Cheesbrough, 2009).

Co-infections of *S. haematobium* and *Salmonella* species can occur, and in the endemic areas the salmonellae are notorious cause of resistant secondary bacterial cystitis (Hathout *et al.*, 1967; Barsoum, 2013). This occurs because there is an established symbiotic association between schistosomes and certain *Salmonella* species (Muniz-Junqueira *et al.*, 2009; Barnhill *et al.*, 2011; Barsoum, 2013). Entero-invasive *Salmonella* enter systemic circulation and attach to integuments of adult *Schistosoma*spp (LoVerde *et al.*, 1980; Melhem and LoVerde, 1984). As such, bactericidal concentrations of antibiotics become largely above the achievable

therapeutic levels of the drugs in co-infected individuals. Chloramphenicol-sensitive *S.* Typhi had beendemonstrated to be refractory to chloramphenicol treatment in co-infection with *Schistosoma*spp (Barnhill *et al.*, 2011)

### 1.1Statement of Research Problem

*Schistosoma haematobium* has been regarded as a 'neglected schistosome' (Rollinson, 2009; Rinaldi *et al.*, 2011; Brindley and Hotez, 2013) despite its implication in HIV/AIDS co-infection and a burden of bladder cancer development. Though most of the infected individuals in endemic areas of Nigeria suffer from light infections, the disease adversely impacts on the economic and general health conditions of the affected communities (Chidozie and Duniyan, 2008; Kanwai *et al.*, 2011). Consequently, the workforce is affected weakness and lethargy, and the academic performance of school children areaffected (WHO, 1999; Uneke *et al.*, 2007, Kanwai *et al.*, 2011). Schistosomiasis can increase the cost and duration of treatment of salmonellosis as *Schistosoma*-associated *Salmonella* evades activities of antibiotics during adherence to the worm.Hence, commonly used anti-*Salmonella* drugs are not useful in co-infected individuals (Barnhill *et al.*, 2011). As outlined by Cheesbrough (2009), high incidence of chronic infections by *S.* Typhi,*S.* Paratyphi A and carrierstates occur in areas endemic for schistosomiasis. The salmonellae colonize the adult schistosome to become protected from antibiotics with resultant immune complex disorder of kidneys (nephrotyphoid) in those with urinary schistosomiasis.

The emergence of antibiotic-resistant *Salmonella* is a great threat to health. Diarrhoea is the leading cause of deaths due to *Salmonella* spp, as both the developing and under-developed nations are faced with foodborne and waterborne outbreaks that have permitted the continuous spread of salmonellosis.

## 1.2Justification

There is a need for routinehealth surveillance on young childrenbecause they are the future of tomorrow and are often involvedin risky juvenile activities that could lead to infections with *Schistosomahaematobium* and *Salmonella* spp.They might be unaware when they become eventually infected. Such risky activities include bathing, swimming, wading and fishing in unprotected or cercariae-infested water bodies. When these infections are undiagnosed or untreated, complications may occur.

This study will assess infection risks associated with use of common sources of water like river or stream by which infected pupils may transmit infectionsto other members of their communities that share the same source of water.

The great challenge of schistosomiasis control is that it is based on snail control, improved sanitation, health education and administration of Praziquantel to pupils or high-risk communities but these are not implemented in all schools and communities. Intermediate snail hosts (*Bulinus africanus*, *Bulinus truncatus* and *Physopsis*spp) that harbour *Schistosoma haematobium*are yet to be controlled in Nigeria;hence the need to decipher the trend of its occurrence for adequate planning of control measures in affected areas of Nigeria.

This study willbring awareness on schistosomiasis and salmonellosis by organizing enlightenment talksin primary schools in Jaba LGA, and generated information will add into growing data on adverse effects and epidemiology of urinary schistosomiasis and salmonellosis.

## 1.3 Aim

The aim of the study was to determine the prevalence of urinary schistosomiasis and its co-infection with *Salmonella* species among pupils in Jaba LGA, Kaduna State.

## 1.4 Objectives

The objectives of this study were to:

1. Determine the prevalence of co-infections of urinary schistosomiasis and *Salmonella* speciesfrom blood and urine among pupils of Jaba LGA of Kaduna State.
2. Determine and compare the packed cell volume (PCV) of pupils with single and co-infections.
3. Determine the occurrences of other Gram-negative uropathogensand their co-infections with urinary schistosomiasisamong the pupils.
4. Assess socio-demographic data and risk factors associated with urinary schistosomiasis and salmonellosisamong the pupils in Jaba LGA.

## 1.5 Research Null Hypothesis ($H_0$)

There is no co-infection of *Schistosoma haematobium* and *Salmonella* species among pupils in Jaba LGA of Kaduna State.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Morphology of *Schistosoma* species

The genus *Schistosoma* is an unsegmented, cylindrical, leaf-like trematode. The adult worms have approximate lengths of 1-2 cm, with two terminal suckers, reproductive organs and blind digestive tract. The males have grooves or gynecophoric channels where the females reside (Leder and Weller, 2011).

### 2.2 History and Epidemiology of Schistosomiasis

After the first isolation of the parasite from human blood vessel in 1851 by a German physician, Theodor Bilharz, the original name of the disease bilharzia (bilharziasis) or Snail fever was later renamed as schistosomiasis (Orihel and Ash, 1995) which is most frequent in literatures today. Schistosomiasis has been termed a "major communicable disease" that impacts greatly on both health and economic statuses of developing countries in the tropics with some associated morbidity and mortality (Mohager *et al.*, 2014).

Schistosomiasis is one of the common parasitic diseases in the world (Gracio *et al.*, 1992; Okpala *et al.*, 2004). It is second only to malaria in tropical diseases and the third in global parasitism (Okwori *et al.*, 2014). Infection with *S. haematobium* has been referred to as 'urogenital schistosomiasis' because it affects veins draining some pelvic organs like the bladder, uterus and cervix. The terminal-spine ova penetrate the tissues and get entrapped or excreted in urine for the propagation of the worm's life cycle (Brindley and Hotez, 2013; Santos *et al.*, 2014; WHO, 2016). However, some hybrids of *S. haematobium* especially *S. bovis, S. intercalatum*, or *S. curassoni* also cause

urinary schistosomiasis. There had been reports of hybrids in Cameroon and Senegal, which were unconfirmed by polymerase chain reaction (PCR) but their egg shape was suggestive of *S. bovis*-*S. haematobium* hybrid (Calvo-cano *et al.*, 2015).

Of all the over 200 million global cases of schistosomiasis, two-third is caused by *S. haematobium* (van der Werf *et al.*, 2003; Brindley and Hotez, 2013). It has been described as an 'ancient scourge' which can be eliminated through the development of new tools, taking advantage of the newly completed genomic sequencing of *S. haematobium* (Brindley and Hotez, 2013).

Schistosomes are a prime example of complex multicellular parasites in human host (Edward and Andrew, 2002). They tend to have specific geographical distribution patterns; *Schistosomamansoni* infection is most prevalent in tropical and subtropical regions including sub-Saharan Africa, the Middle East, South America and the Caribbean (Leder and Weller, 2011; Barakat, 2013; Elbaz and Esmat, 2013). *Schistosoma haematobium* infection is mostly found in North Africa, the Middle East, Turkey and India. *Schistosomajaponicum* occurs in Asian countries, especially China, Philippines, Thailand and Indonesia (Leder and Weller, 2011; Barakat, 2013). *Schistosomaintercalatum* and related *Schistosoma guineensis* are distributed in rain-forest regions Central and West Africa; whereas *Schistosomamekongi* is restricted to Cambodia and Lao Peoples' Democratic Republic (Leder and Weller, 2011; Barakat, 2013).

Schistosomiasis occurs in 74 developing tropical and subtropical countries (WHO, 2010; Barnhill *et al.*, 2011), but the trend is changing. From being endemic in 77 countries (WHO, 2012), it has increased to 78 countries in which over 200 million individuals are infected (WHO, 2013a; Mohager *et al.*, 2014) and 90% of the global

cases occurred in Africa (Brindley and Hotez, 2013). The disease has remained one of major public health problems of humanity with severity on both economic and social development (WHO, 1999; Kanwai *et al.*, 2011). There has been an alarming estimate of 201.5 million *Schistosoma* infections (mainly *S. haematobium*) to occur in Africa (and accounting for more than 97% of the estimated number of infections worldwide). A considerable morbidity and mortality have been associated to the developing world (Hotez and Fenwick, 2009) while its distribution is peculiar to the ecology that permits its transmission (SarkinFada *et al.*, 2009).

### 2.2.1 Life cycle and Transmission of *Schistosoma* species

The life cycle (Figure 2.1) of the genus *Schistosoma* is a complex one, requiring both intermediate and definitive hosts (Leder and Weller, 2011; CDC, 2012b; Mohager *et al.*, 2014). A female produces hundreds to thousands of eggs per day which are voided in urine (or stool) into fresh water and later hatch to release ciliated miracidia. The miracidia penetrate intermediate (snail) host; and through a period of 4-6 weeks they asexually multiply into many sporocysts that develop into cercarial larvae; and subsequently leave the body of the snail host as bifurcated-tailed cercariae (CDC, 2012b). The cercariae are attracted by body's warmth and skin lipids (Sakanari and Mckerrow, 2010) to locate their definitive (human) host during water-contact activities. However, schistosomes have been found in various animals including birds, dogs, cats, rodents, pigs, horses and goats. After penetrating the human skin and losing their forked-tail, they change into schistosomulae (Garcia, 1999, Leder and Weller, 2012, CDC, 2012b). The schistosomulae migrate via blood and lymph to mesenteric arteries, splanchnic arteries or portal veins and develop into adult worms. In the definitive host,

the adult worms lay eggs again which are voided in urine (or stool) in to fresh water, and the life cycle continues (Leder and Weller, 2011; CDC, 2012b).

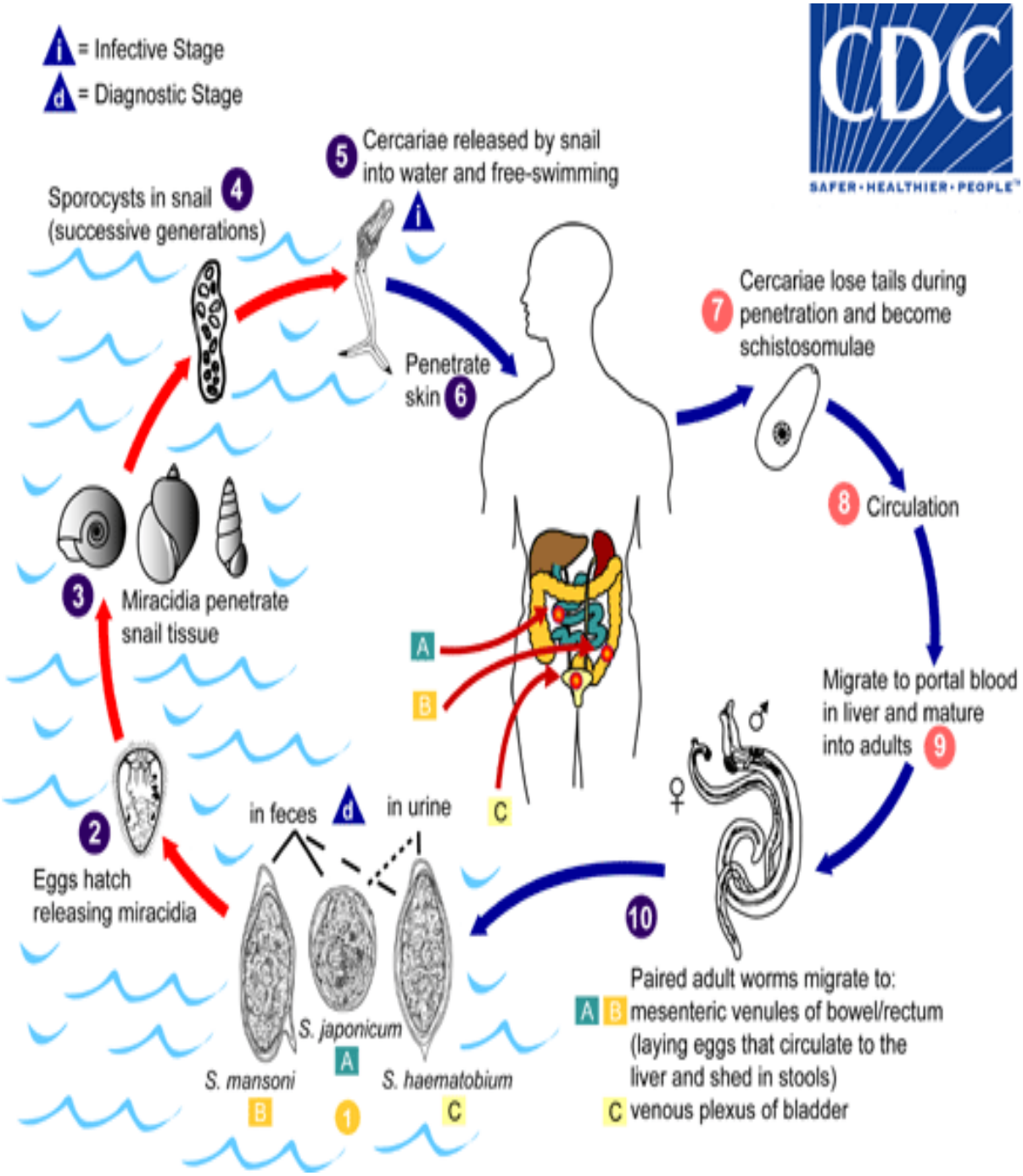


**Figure 2.1: Life cycle of *Schistosoma* species**

**(Source: CDC, 2012b).**

## 2.3 Risk Factors and Demographics Associated with Schistosomiasis

As demonstrated by Kanwai *et al.* (2011), schistosomiasis decreases with increase in age. This means children are most prone to the infection. In a study by Mohager *et al.* (2014), some of the risk factors assessed for schistosomiasis indicated that low awareness of the disease, contact with unsafe water during swimming, washing of clothes and utensils in/with such water, and undertaking agricultural activities barefootedenhancedthe risk of contracting schistosomiasis.

Male subjects are more affected with schistosomiasis than female subjects especiallyin the age-group 11-15yrs (Omenesa *et al.*, 2015) because of their propensity to indulge in swimming in canals and other stagnant water bodies, fishing, rice farming and irrigation scheme (Mohager *et al.*, 2014).

The endemicity of schistosomiasis in Africa and the Middle East is due inadequate sanitary practices and lack of safe water system (Steinmann *et al.*, 2006). Children (especially in public) primary schools indulge in indiscriminate water activities in unprotected snail/cercariae-infested water bodies (Ibironke *et al.*, 2012). Since schistosomiasis statistically decreases with increase in age (Kanwai *et al.*, 2011), it indicates therefore that children are most prone to the disease. This is the case in Nigeria as it is one of Africa's highly endemic countries (Chitsulo *et al.*, 2000; Adeyeba *et al.*, 2002).

## 2.4 Clinical Presentations of Schistosomiasis

Schistosomiasis is a chronic debilitating disease affecting human population (Noble and Glem, 1982) caused by *Schistosoma* sppand it is not an asymptomatic infection. It has been re-emphasized by King (2015) that schistosomiasis in its nature is truly a chronic inflammatory disease of the intestines, urinary tract or organs since eggs of *Schistosoma* spp cause inflammation and damage of tissues immune-mediated inflammations. Schistosomiasis causes economic and health burdens by disabling a sufferer, as well as leads to death of between 14,000 (Mohager *et al.,* 2014) to 200,000 per year (WHO, 2016).

The term schistosomiasis refers to any of the two forms: intestinal schistosomiasis caused by *S. mansoni, S. japonicum,* as well as *S. mekongi* and *S. intercalatum,* which are human-adapted species; or urinary schistosomiasis caused by *S. haematobium* (Jamjoom, 2006). However, intestinal schistosomiasis is mainly caused by *S. mansoni*, it has been reportedly caused also by *S. haematobium* (Elbaz and Esmat, 2013), and hepatosplenomegaly is evidenced when it becomes chronic (Grimes *et al.*, 2015).

Usually, most people at the time of infection present with no symptoms (CDC, 2012b) butclinically, people from endemic areas tend to have less reaction because of previous exposure than travelers from non-endemic areas. Symptoms can be localized (e.g. in the bladder) or generalized (e.g.fatigue), but chronic complications require heavy infection (Leder and Weller, 2011). Haematuria along with proteinuria is associated with the disease (Cheesbrough, 2009, Leder and Weller, 2011).

Acute *Schistosoma* infection may present as swimmer's itch or Katayama fever. The former is a localized dermatitis that may result into a pruritic papular or urticarial rash at the initial site of cercarial penetration on exposed skin of the legs or feet. Pruritic papular eruption can intensify within 12-24 hrs; this rarely occurs with *S. haematobium*

except *S. japonicum* (Leder and Weller, 2011). Katayama fever ensues as a result of hypersensitive systemic reactions to migrating parasites in blood between 2-8 weeks post exposure. It is infrequent with *S. haematobium* infection, but is observed during infection with *S. mansoni*or*S. japonicum*. Certain symptoms have been identified with Katayama fever that include abrupt onset of fever, chills, myalgias (muscles pain), arthralgias (joint pain), dry cough, (chronic) diarrhoea and headache, lymhadenopathy, hepatosplenomegaly, eosinophilia, patchy infiltrate in lungs, weight loss, dyspnea (Leder and Weller, 2011, CDC, 2012b). These symptoms are self-limiting but neck stiffness, coma and occasional deaths are possible (Leder and Weller, 2011; Mohager *et al.*, 2014).

Though *S. haematobium* has special preference for venus plexuses of the bladder, it is not rare to find them in ectopic extra-vesical sites like the liver and appendix. This is due to transfer of fertilized eggs along the blood stream to those sites. A recent case report from Kano-Nigeria indicated *S. haematobium* in cutaneous (i.e. skin) schistosomiasis on the neck and back of young adult male with pruritic papular lesions. Such cases would require more diagnosis by histology of skin biopsies (Atanda *et al.*, 2012).

### 2.4.1 Complications of Schistosomiasis

Endemic areas are known to have more cases of chronic complications of schistosomiasis usually associated with heavy infection, large number of tissue-entrapped eggs, their anatomic site distribution, duration and intensity (Leder and Weller, 2011).

More serious complications ensue upon healing many years after than with active pathology in schistosomiasis, which may include fibrotic lesions, glomerulonephritis,

amyloidosis and malignancy or cancer (Elbaz and Esmat, 2013). Chronic schistosomiasis is a risk factor for nontyphi *Salmonella* infection and promotes development of squamous cell bladder cancer (Cheesbrough, 2009). Granulomata due tourinary schistosomiasis following healing are often associated with sandy patches or confluent fibrosis and calcification. This condition can affect bladder muscles and the ureteric walls with consequent bladder dysfunction, disruption in internal vesical or ureterovesical sphincter mechanism (Elbaz and Esmat, 2013). Urinary schistosomiasis exacerbates *Salmonella* infections and carrier states due to *S.* Typhi and *S.* Paratyphi. Chronic urinary schistosomiasis causes calculi (stones) in urinary tracts and bladders of the patients. Anaemia and impairments of growth/development are also associated with urinary schistosomiasis (Cheesbrough, 2006). Portal hypertension and central nervous system complications can occur due to schistosomiasis (Leder and Weller, 2011).

Aside *S. haematobium* being the cause of urinary schistosomiasis, it is incriminated as an emerging co-factor in Africa's AIDS and cancer epidemics (Brindley and Hotez, 2013). The nature of damage to bladder, ureters and kidneys is progressive (Baberjee and Agrawal, 1992). The bladder deformities are due to ureteric inflammation, urinary secondary bacterial infections, hydronephrosis and consequent renal failures in millions of Africans (Botelho *et al.*, 2011; Botelho *et al.*, 2015).

One serious complication of schistosomiasis is female genital schistosomiasis (FGS). The female cervix, fallopian tubes, and vagina are the common gynaecological sites prone to infection with *S. haematobium.* Immunologic responses to both dead and viable eggs lead to lesions, with increased risk of transmitting HIV (Kjetland *et al.*, 2012), painful sexual intercourse, vaginal bleeding and nodules on vulvae (WHO, 2016). Though very rarely, the eggs may be found in the brain or spinal cord which can cause seizures, paralysis, or inflammation of the spinal cord (CDC, 2012b). Another rare

complication is schistosomal appendicitis in intestinal schistosomiasis that had occurred both in endemic underdeveloped and developed countries. It is due to blockage of the appendiceal lumen by adult schistosomes and not from their egg deposition (Elbaz and Esmat, 2013). Among infected males, the disease induces pathologies in their seminal vesicles and prostate glands (WHO, 2016).

### 2.5 Diagnosis and Identification of Urinary Schistosomiasis

Microscopic detection of *S. haematobium* ova in urine remains the gold standard for diagnosis of urinary schistosomiasis (Ibironke *et al.*, 2011; Barsoum, 2013). The ova measure 112 to 170 µm by 40 to 70µm with characteristic yellow-brown colour anddistinct terminal spines (Garcia, 1999). Cytoscopic examinations are totally unnecessary in endemic areas, but will reveal the presence of any lesion in the bladder (Barsoum, 2013).

The definitive diagnosis of schistosomiasis is by egg identification using microscopy; however, serology and radiology, and other non-specific markers (like eosinophilia, thrombocytopenia, anaemia) are useful, but concentration method helps to increase the sensitivity of microscopy. Detection of IgG anti-*Schistosomamansoni* by ELISA technique is a useful tool and the use of urine dipstick diagnostic test can detect schistosome circulating cathodic antigen (CCA). Biopsy specimens (from rectum, intestines and liver) can be screened for schistosome eggs (Elbaz and Esmat, 2013).

Detection of free circulating schistosome DNA by PCR can be used as a valuable test for early diagnosis of prepatent schistosomiasis infection (Hussein *et al.*, 2012, Gomes *et al.*, 2014) but this is largely by chance because only a small volume of processed specimen is used which may or may not contain egg(s). Hence, PCR does not provide a significant clinical benefit because it is faced with the same limitation as microscopy

(Wichmann *et al.*, 2009). Similarly, one of the major setbacks of diagnosis of schistosomiasis by serology lies on its inability to distinguish between current and past infection states with prolonged positive period in an already infected individual. However, a negative test in an area endemic for schistosomiasis isindicative of its elimination (Elbaz and Esmat, 2013).

**2.6 Immunityagainst Schistosomes**

Schistosomes flourish in human hosts despitethe presence of immune responses (Edward and Andrew, 2002). The host immune responses in schistosomiasis are complexbecause the adult worms absorb host's proteins to coat their integuments (Leder and Weller, 2011) which enable them to partially evade host defenses (Cox, 2004). In this way the adult worms are able to live and reproduce many years in a host who then becomes relatively resistant to new infection (Cox, 2004). There are both innate and acquired immunities to schistosomiasis. Though the innate immunity is poorly understood, host's genetic traits had led to the development of heavy infections in some individuals; whereas others suffered only minimal worm burdens. Human adults also develop faster immunity than children (Leder and Weller, 2011).

However, clinical disease is due toimmune reaction by type-2 T-helper cells (Th2) against invading eggs in tissues. This leads to fibro-granulomatous inflammation and activation of mediators of fibrosis (i.e., hepatic stellate cells). Host's cell-mediated immune response to soluble egg antigen of *S. mansoni* cause granulomata in the liver;it extends into an irreversible fibrosis and portal hypertension (Elbaz and Esmat, 2013). In an area endemic for schistosomiasis, previous infections make a patient to react less than people without previous history of the disease and from non-endemic areas (Leder and Weller, 2011).

## 2.7 Control and Prevention of Schistosomiasis

Despites decades of WHO's implementation of control programmes for schistosomiasis in endemic countries, eradication in many countries has not been achieved. A few countries like Japan and Egypthaveeradicated the disease, yet 230-240 million people globally are still infected (Barsoum *et al.*, 2013; Gomes *et al.*, 2014, WHO, 2016), and are capable of transmitting more infections to othervulnerablepeople and communities.

In the face of pronounced evidence of Praziquantel efficacy, its safety and benefit in the treatment and control of schistosomiasis, the drug is still inaccessible formany young children; hence, creating a significant treatment gap (Stothard *et al.*, 2013) especially in the underdeveloped nations.

Control of schistosomiasis has been based on administration of Praziquantel to school-age children and high-risk communities (Fenwick and Webster, 2006; Nahum *et al.*, 2012; King and Bertsch, 2013);however, the populations of intermediate hosts (*Bulinus africanus, B. truncatus, B. tropica* and *Physopsis* spp) in the tropics and subtropics have not beencontrolled (Assafa *et al.*, 2004). They serve as sources of new infections that can increase the morbidity of schistosomiasis among the swimming population of a community, especiallyin stagnant water. It is important to bring an awareness of schistosomiasis to rural areaswith populations that constantly drift into the cities and infected individuals may transmit new infections. Schistosomiasis education can be included in the curricula of primary school since children are the most prone. Brindley and Hotez (2013) hadoutlined what are needful for elimination of the disease: development of new diagnostic tools, drugs, vaccines and early reporting of clinical and

research findings. There is need for eradication of the intermediate (snail) hosts of schistosomes from lakes with concerted efforts in educating the pupils on the dangers of exposing themselves to unprotected water bodies (Omenesa *et al.*, 2015). Improved access to clean water and adequate sanitation can reduce the risk and transmission of schistosomiasis. This is achievable by use of soap, detergent and endod which might kill snails and the cercariae that emerge from them (Grimes *et al.*, 2014).

### 2.8*Salmonella* species

*Salmonella*belongs to the family Enterobacteriaceae and has a very complex nomenclature. Following molecular studies (of DNA homology)only two species in the genus *Salmonella* exist: *Salmonella bongori* and *Salmonella enterica* (which includes the following six subspecies: I = *enterica,* II = *salamae*, IIIa = *arizonae,* IIIb = *diarizonae*, IV = *houtenae*, and VI = *indica*) (UK SMI, 2015).

These Gram-negative bacteria are rod-shaped, non-spore-forming and predominantly motile by means of peritrichous flagella. They range in diameters of about 0.7-1.5μm and lengths of 2-5μm with a few exceptions. They are generally lactose non-fermenters and obtain energy from oxidation and reduction reactions using organic sources and anaerobically facultative. They produce acid and gas from glucose and are oxidase negative. Hydrogen sulphide ($H_2S$) is produced by most of them with exceptions of *S.*Paratyphi A, and *S.*Typhi –which is a weak producer(UK SMI, 2015).They are identified by a combination of biochemical and serological tests (PHLN, 2000;UK SMI, 2015).

In view of the new nomenclature of this bacterium, some laboratories may continue to report serotypes as species for some time to come. But its nomenclature continues to become complex as new serotypes are discovered each year(UK SMI, 2015).

### 2.8.1 Salmonellosis

Epidemics due to *S. enterica* had involved distribution of predominant epidemic strains over large geographical areas.However,*S.* Typhimurium serovar is the common cause of salmonellosis in humans and animals (Ikuo *et al.*, 2011); it has been described the second most occurring serovar of *Salmonella* in human disease (CDC, 2007). Occurrence of *Salmonella* infections still continues at high frequencies in both industrialized and developing nation (Salehi *et al.*, 2005).*Salmonella* Typhi with many other nontyphoidal serotypes (NTS)of *S. enterica* are important aetiologies of childhood bacteraemia among African children (Graham, 2002; Morpeth *et al.*, 2009).Cases of salmonellosis hadbeen associatedwith direct and indirect contact with reptiles and amphibians(Mermin *et al.*, 2004).

Salmonellosis is one of the commonest and widely distributed foodborne diseases in the world.Tens of millions of human cases occureach year with overa hundred thousand deaths. The pathogen, *Salmonella*,has a ubiquitous distribution;it is described as a 'hardy bacterium' capable of surviving several weeks in dry environments and for several months in water. All serotypes of *Salmonella* cause human diseases.However, animal host-specificity is observed in *Salmonella* Dublin (in cattle) and *Salmonella* Choleraesuis (in pigs); they are invasive and life-threatening in human cases(WHO, 2013b).

Anyone can contract salmonellosis provided there is consumption of *Salmonella*-contaminated food (like egg, poultry meat, egg products and dairy products, water or direct contact with patients or carriers) (Lawley, 2013). Children(particularly the younger ones), the elderly, patients with low gastric acid (i.e., hypoacidity) or patients

with immunodeficienciesare at higher risk of salmonellosis (Cianflone, 2008; Hoelzer *et al.*, 2011; CDC, 2012a).

### 2.8.2Clinical presentations of salmonellosis

The term salmonellosis is a general name given to the syndromes caused by salmonellae (Mirmomeni *et al.*, 2009). *Salmonella* is a primary inhabitant of the gastrointestinal tractand a common waterborne and foodborne cause of gastrointestinal and systemic diseases worldwide. Itis the leading cause of deaths of approximately 2 million people each year due to diarrhoea (Mohager *et al.*, 2014). Nontyphoidal salmonellosis is common in most parts of the world, but is widespread in Europe, North America, Latin America, the Middle East, India, Japan, United States and in Africa (Abdullahi *et al.*, 2015).

*Salmonella,* as an intracellular anaerobe, causes a wide spectrum of diseases that include gastroenteritis, enteric fever, bacteraemia, focal infections and convalescent lifetime carrier state. The type of infection caused depends on the serotype of *Salmonella* and certain host factors (Owens and Warren, 2015).

Salmonellosis caused by *Salmonella* Enteritidis is the most common bacterial foodborne disease in the United States (Linam and Gerber, 2007).*Salmonella* has a widespread distribution in the environment, but certain host factors make humans a particular susceptible species (Owens and Warren, 2015). There are nine most common human serovarsof *S. enterica* responsible for more than 60% of human salmonellosis cases based on the Centers for Disease Control and Preventions (CDC) annual summary of 2005: *Salmonella* serovars Typhimurium, Enteritidis, Newport, Heidelberg, Javiana, Montevideo, Muechen and Saintpaul (CDC, 2008; Abakpa, 2014). Two distinct syndromes are caused by *Salmonella*:enteric (typhoid and paratyphoid) fever and gastroenteritis (PHLN, 2000).

Generally, patients with salmonellosis may present with some or all of the following symptoms: vomiting, diarrhoea, headache, abdominal pain, body ache, breathlessness, weight loss, constipation and anaemia (Abdullahi *et al.*, 2015). Chronic salmonellosis presents with severe anaemia, significant hepatosplenomegaly, mild lymphoadenopathy, eosinophilia but no leukocytosis. Other symptoms like haematuria, petechiae, epistaxis (nosebleed), proteinuria, and purpuric lesions may be identified (Mohager *et al.*, 2014).

**a.Gastroenteritis**

Bacterial gastroenteritis, commonly called bacterial diarrhoea, bacterial enteritis or infectious diarrhoea is caused by different pathogens that include *Salmonella*spp, *Shigella*spp, *Vibrio*spp, *Campylobacter*spp, *Plesiomonas*sppand *Escherichia coli* (Mikoleit, 2010). However, *Salmonella*sppand *Shigella*sppare the two most common causes of bacterial gastroenteritis (Procop *et al.*, 2008; Mikoleit, 2010).

Enteritis is the most common form of salmonellosis during foodborne outbreaks and sporadic cases of the disease thatcan result from the ingestion of contaminated poultry meat and eggs with incubation period of 6-72hours. Its common symptoms include nausea, vomiting, non-bloody diarrhoea, fever, cramps, myalgia and headache caused by *S.* Enteritidis (WHO, 2013b).Gastroenteritiscan also be caused by over 2,300 serotypes of salmonellae other than *S.* Typhi, *S.* Paratyphi A,*S.* Paratyphi B and *S.* Paratyphi C (PHLN, 2000).

**b.Enteric Fever and Bacteraemia**

Enteric fever means typhoid fever. Bothtyphoid and paratyphoid fevers are commonly grouped together as enteric fever (PHLN, 2000; White, 2010).*Salmonella* Typhi and Paratyphi are major causes of entericfever in underdeveloped countries mainly due

toimproper sewagedisposal and water treatment (Procop *et al.*, 2008; Mohager *et al.*, 2014).Enteric fever is a human-specific disease that is primarily caused by *Salmonella* Typhi. About 21 million new infections per year occur with over 200,000 deaths worldwide (Gonzalez-Escobedo *et al.*, 2011).It is a generalized infection of the reticuloendothelial system and intestinal tissue;usuallyit is accompanied by fever and bacteraemia (PHLN, 2000).

Bacteraemia occurs when bacteria invade the blood either transiently or persistently and several populations are at high risk of bacteraemia especially the infants, the elderly and immunocompromised persons. Bacteraemia is a common complication of *Salmonella* gastroenteritis (Cianflone, 2008).

**c.Carrier State in Asymptomatic Individuals**

Chronic carrier states occur in 3-5% of people infected with *S.* Typhi, and mostly in patients that have recovered from the acute phase of typhoid fever. The bacterium uses the gallbladder as reservoir for the infection(Gonzalez-Escobedo *et al.,* 2011). There can be continuous shedding of *Salmonella* in faeces of infected humans for a period of timeafter subsidence of symptoms. Some of these individuals become chronic carriers (Lawley, 2013).A very popular historic example of carrier state was Mary Mallon (or Typhoid Mary), a cook in New York City (USA) in the early twentieth century (Gonzalez-Escobedo *et al.*, 2011). Typhoid carriers excrete *S.* Typhi in urine for many years, and are common in areas endemic for schistosomiasis (Cheesbrough, 2006).

**2.8.3Diagnosis of Salmonellosis**

Widal test is most commonly used for serodiagnosis of enteric fever caused by *Salmonella*spp. The test measures agglutinating antibodies to the O and H antigens of

*Salmonella* Typhi. Although some serodiagnostic techniques have been developed for the detection of antibodies to outer membrane proteins, lipopolysaccharide and flagellin protein; they are not routinely used or recommended for the diagnosis of salmonellosis (PHLN, 2000).

Widal test, although positive in 25% of cases, is still not helpful in the diagnosis of the salmonellosis (Lambertucci *et al.*, 1998). Sensitive and specific laboratory methods for the isolation, identification and serotyping of *Salmonella*are vitalfor monitoring and control programmes. The ideal diagnostic test for *Salmonella*should be rapid, inexpensive, easily reproducible, sensitive, and specific; however,there is currently, no single method that meets all these criteria. Molecular methodologies can increase sensitivity,specificity and reducetime wasting, but traditional culture methods remain the diagnostic standard for identification of *Salmonella* species (Mikoleit, 2010). Beyond the routine of biochemical and complete serological identification of *Salmonella*spp, some reference laboratories carryout other specific subtyping; for example, biotyping, phage typing, molecular finger printing (PHLN, 2000).

Theoretically, traditional culture is 100% specific and provides the investigator with an actual isolate. Pure isolates are crucial for antimicrobial susceptibility testing and most subtyping methods like serotyping and Pulse Field Gel Electrophoresis (Mikoleit, 2010). For laboratory investigation during suspected outbreaks of gastroenteritis, blood, urine, faeces, rectal swab, food and water samples can be examined immediately or stored at $4^{o}$C for 24hours; or byusing Modified Stuart's Transport Medium,samples can be kept for 24 hours at room temperature (PHLN, 2000).

*Salmonella* Typhiis detected more frequently (at 70.7%) than other salmonellae because of its higher frequencyin typhoid fever among schistosomiasis patients than the

paratyphoid fevers (Mohager *et al.*, 2014). The isolation of *Salmonella*is always significant but a negative culture does not exclude the diagnosis of salmonellosis (PHLN, 2000).

**a.Selective media for Isolation of *Salmonella* spp**

The use of selective and/or differential media suppresses the growth of other faecal-flora while allowing the growth of targeted (*Salmonella*) pathogens (Mikoleit, 2010).Enrichment is performed ineither Selenite-F broth (Mikoleit, 2010) orThioglycollate broth (Abdullahi *et al.*, 2015), Tetrathionate broth (PHLN, 2000) for 18-24hrs at 37$^{o}$C and cultured on any of the following media: 5% Sheep Blood Agar (or substituted with another non-selective media like Mueller-Hinton agar), MacConkey (MAC) agar, Hektoen Enteric (HE) agar or Xylose-Lysine-Desoxycholate (XLD) agar (Mikoleit, 2010). Others like Deoxycholate Citrateagar, Brilliant Green Agar, Cystine-lactose-electrolyte deficient (CLED) agar andmany other commercially validated media can be used (UK SMI, 2015).

However, HE or XLD are recommended, which choicesdepend on their quality and availably in a given region (Mikoleit, 2010); they allow isolation of *Shigella*sppalso (PHLN, 2000).On XLD, majority of *Salmonella* colonies range from clear to pink or red. Most colonies are 2-4 mm in diameter with black centers (Mikoleit, 2010). Following an aerobic incubation at 35–37°C for 18-24hrs of *Salmonella*-inoculated XLD plate, the colonies appear red, and usually with black centers (but absent inserotypes like *S.*Paratyphi A and *S.*Typhi) (UK SMI, 2015). *Salmonella* grows on *Salmonella-Shigella* Agar(SSA)as anon-lactose fermenter with bleached or fogged colonies and some black points (Mirmomeni *et al.*, 2009). However, MAC has low selectivity than XLD, SSA and HE, which are intermediately selective. For

highselectivity, Bismuth Sulphite Agar (BSA) and BGA may be used. The BSA, XLD and HE contain $H_2S$ indicator systems necessary for the detection of *Salmonella* (especially *S. enterica* subspecies IIIa and IIIb) which ferment lactose and other salmonellae from dairy factory environments(PHLN, 2000).

**b.Isolation of *Salmonella* spp from Blood Samples**

*Salmonella* sppare easily recovered from blood of patients with increasing eosinophilia after treatment with antibiotics (Lambertucci *et al.,* 1998). It is only recently that closer attention had been drawn to isolation of *Salmonella*sppfrom blood. Most researches had centered on stool isolation of the pathogen (Abdullahi *et al.,* 2015).

Due to continual increase in multiple antibacterial drug resistance, determination of antibiotic resistance along with early diagnosis of *Salmonella* infections has become a matter of vital importance. Blood cultures are routinely used for recovery of isolates, which are further testedfor susceptibility to antibacterial drugs (Abdullahi *et al.,* 2015).

Blood culture is probably the single most useful diagnostic procedure for diagnosis of enteric fever. With a few exceptions, a positive blood culture has a high predictive value for current enteric fever. Although bone marrow has high positive yielding capacity, sampling of marrow is not often performed because it requires high skills and sterile equipment, and it is often unpleasant for the patients (Vallenas *et al.,* 1985; Cheesbrough, 2009; PHLN, 2000). Therecovery of an isolate from a blood sample that biochemically resembles a *Salmonella*sprequires furtherconfirmation by serotyping (PHLN, 2000).

### c.Isolation of *Salmonella* spp from UrineandStool Samples

In 25% of cases of salmonellosis, *Salmonella* had been isolated from faeces or urine of the patients (Lambertucci *et al.,* 1998). However, isolation from urine is rare (Singh *et al.*, 2011) even in the endemic areas (Pegues and Miller, 2009).Most laboratories prefer to examine faecal samples in the diagnosis of (*Salmonella*) diarrhoeal diseases, but it is complicated by multiple factors that include previous antibiotic treatment, transport stress, intermittent shedding of pathogens in the faeces, and low numbers of *Salmonella* in relation to other enteric flora. Hence, there is the need to use culture procedures that employ selective enrichment and the use of selective or differential media (Mikoleit, 2010).

## 2.9 *Salmonella* in Public Heath

*Salmonella* species are become a problem to human health. They are continually been spread through food and water. Control of *Salmonella* transmission will help in preventing human and animal infections, because they are becoming resistant to available antibiotics, especially when they attach to integuments of schistosomes (Barnhill *et al.,* 2011).

### 2.9.1 Transmission of *Salmonella*

In the face of improvements in global health facility, the problem of bacterial infections remains.*Salmonella* sppare well transmitted via food as the most important foodborne pathogens (Mirmomeni *et al.*, 2009), as well as via water (Mohager *et al.*, 2014). Of all the species, *S. enterica* subsp. *enterica* causes 99.5% of all human and animal infections and often zoonotic. However, eggs and poultry meat have been implicated as the main reservoir of salmonellosis due to*S*. Enteritidis (Marcus, 2007).Of all the outbreaks of

salmonellosis caused by *S.* Enteritidis in the United States each year, 80% were due to consumption of egg (Braden, 2006). There are many reservoirs of *Salmonella* sppwhich include humans, poultry, swine, cattle, rodents, and pets such as iguanas, tortoises, turtles, terrapins, chickens, dogs, and cats. *Salmonella is* harboured in gastrointestinal tracts of about 90% of reptiles and amphibians, whereas about 6% of nontyphoid diseaseshad been related to direct contact with these animals (Linam and Gerber, 2007).

Marketing of animal/poultry-derived fast foods in domestic and international trades have facilitated the spread of salmonellosis, butits continuous distribution within the environment occurs due to faecal contaminationof water and food (Mirmomeni *et al.*, 2009).However, WHO (2013b) outlined five major sources and transmission routes of *Salmonella* to include domestic and wild animals, entire food chain, contaminated food of animal origin, person-to-person, contact with infected animals or pets.

### 2.9.2 Antibiotic Resistance of *Salmonella*

There is an outcry of increasing antibacterial drug-resistance in *Salmonella* species. Their virulence and adaptability constitute a major problem for public health worldwide. This similar situation had led to an increased treatment failures with the usage of empirical therapy in recent times among patients with salmonellosis (Meneses, 2010; Abdullahi *et al.*, 2015; Owens and Warren, 2015). The antibiotic resistant genes are exchanged via transferable plasmids among the bacterial population (Abdullahi *et al.*, 2015).

### 2.9.3*Schistosoma-Salmonella* Co-infection/Association in Human Host

There are growing evidences suggesting the bacteria-parasite interactions especially between *S.* Typhior *S.* Paratyphiand schistosomiasis. All *Schistosoma* species can be

found in co-infections with over 20 *Salmonella* species of both human and animal origins (Muniz-Junqueira, 2009, Barnhill *et al.*, 2011, Mohager *et al.*, 2014).

In areas endemic for schistosomiasis, co-infection of *Salmonella* and *Schistosoma* species presents a synergistic bacteria-parasite interaction and patients with the dual infections show typical symptoms of typhoid fever (Igwe and Agbo, 2014) which is often complicated (Barnhill *et al.*, 2011). Although the two pathogens enter the body through separate routes (oral versus dermo-invasion), they meet in systemic circulation in the portal mesenteric system to exert this interaction (Melhem and LoVerde, 1984). The major problem as a result of the interaction is that after the binding of salmonellae to the integuments of adult schistosomes using fimbrial protein (FimH), they multiply and become resistant to antibiotics (Barnhill *et al.*, 2011; Mohager *et al.*, 2014). This leads to protracted *Salmonella* infection which is both difficult to diagnose and to treat, especially when the underlying schistosomiasis has lasted over a year in children than adults (Mohager *et al.*, 2014). However, both persistent and recurrent *Salmonella* bacteraemia and schistosomiasis have been described in children and adults (Mohager *et al.*, 2014).

Chronic or persistent *Salmonella* bacteraemia in co-infection with *Schistosoma mansoni* presents with symptoms that involve indolent febrile disease, lasting for several weeks to a few years (Lambertucci *et al.*, 1998). The fever is high which could be irregular, continuous or intermittent and often accompanied by headache, sweating, chills, weight loss, diarrhoea, abdominal pain and bacteraemia with either one or more species of *Salmonella*. Also this co-infection with *Salmonella* occurs more frequently with urinary schistosomiasis than intestinal schistosomiasis (Mohager *et al.*, 2014).

*Salmonella-Schistosoma* co-infected patients in the population often experience fatigue, malaise, weight loss and fever. Although the bacteraemia could last for many months, the patients do not have evidence of toxaemia (Lambertucci *et al.,* 1998). Many other patients with the hepato-intestinal form can present with similar syndrome. Oedema and petechial rashes on the lower limbs are common, but prostration, delirium and localized infections are not seen (Lambertucci *et al.,* 1998).

The salmonellae have been found on the integuments or in the intestinal tracts of adult worms of *S. mansoni* (Lambertucci *et al.,* 1998; Mohager *et al.,* 2014) showing that schistosomes are a source/vehicle for transfer of *Salmonella* (LoVerde *et al.,* 1980). The resulting effect is a reduction of antibody activity against *S.* Typhiand *S.*Cholerasuis in sera of patients with hepato-splenic schistosomiasis as compared to normal controls (Lambertucci *et al.,* 1998).

The co-infection has rare mortality but antibiotic therapy is less effective. Recurrent *Salmonella* bacteraemia is common and continues when underlying schistosomiasis is untreated (Lambertucci *et al.,* 1998). Hence, effective treatment of schistosomiasis alone deprives the *Salmonella* of favourable foci for growth, and eliminates both infections in more than 90% of the cases (Lambertucci *et al.* 1998).

### 2.9.4Control and Prevention of Salmonellosis

Occurrences andspread of antibacterial drug-resistance is complex;majorlywidespread use of antibacterial drugs in foodanimals, particularly in animal feed is implicated (Abdullahi *et al.,* 2015). Hence, controlled usage ofantibacterial drugs in food animals will help to reduce this complex problem.Basic food hygiene practices have been recommended by the WHO as a preventive measure, especially thorough cooking of food (WHO, 2013b) as well asproper sewage control and supply ofpotable water.

# CHAPTER THREE

## 3.0MATERIALS AND METHODS

### 3.1 Study Area

The study was conducted in some selectedpublic primary schools in Jaba Local Government Area (LGA), Kaduna State, Nigeria.Jaba is located in the Northern hemisphereonLatitude 9° 19' 47"N to Latitude 9° 36' 35"N, and in theEast on Longitude 7° 56' 24"E to Longitude8° 12' 36"E (Fig. 2). The area is occupied by the Ham People, who are notable for the rich Nok culture.Archeologists discovered the Nok Terracottafrom Jaba which was carbon-dated to 2000-2500 years ago. "Kwain" (or Kwoi which means "Community of the United") serves as the political capital of the LGA (Nokculture, n.d).

Jaba LGA has many villages that include Bitaro, Nok, Kwoi, Zshiek (Kurmin Musa), Dung (also called Jaban Kogo), Chori, Fai, Ketere, Sambang Gida, Sambang Daji, Dura, Ankun, Gora, Kurmi Danagana, Tunga and many other Ham settlements in the Southern parts of Kaduna State (Nokculture, n.d).The people of the area are predominantly farmers; they cultivate large quantities of ginger, *Digitariaexilis*, (popularly called 'acha'or 'hungry rice'), cocoyam, guinea corn, millet and maize among many others.

### 3.2Study Design

Ten villages, namely, Ankun, Bitaro, Central Area, Chori, Dura, Gora, Kwoi, Nok, Sambang and Yadi-Pyok were randomly selected from Jaba LGA, Kaduna State, Nigeria. The study was cross-sectional. Simple random sampling method was used to select the subjects from among the volunteers in each class of the participated schools from each of the villages.

### 3.3 Ethical Approval and Consent

For this study, ethical approvals (in Appendices V-VI) were obtained from the Kaduna State Ministry of Health (Ref: MOH/ADM/744/VOL.1/290) and Kaduna State Universal Basic Education Board (KDSUBED) (Ref: KD/SUBEB/380/VOL.1/8). Photocopies and originals of these approval letters were presented at the Education Office ofJaba LGA of Kaduna State where subsequent endorsement letters were issued to the Heads of each of the randomly selected primary schools within the LGA. Ten public primary schools (one from each village) were selected.

In every selected school, awareness talks on schistosomiasis, its danger, transmission, control and prevention were delivered. The pupils were informed of the need to volunteer to be part of this study by willingly submitting their urine and blood samples for laboratory diagnoses. Those pupils that volunteered to be part of the study were given consent forms to present to their parents/guardians for full permission to enroll them.

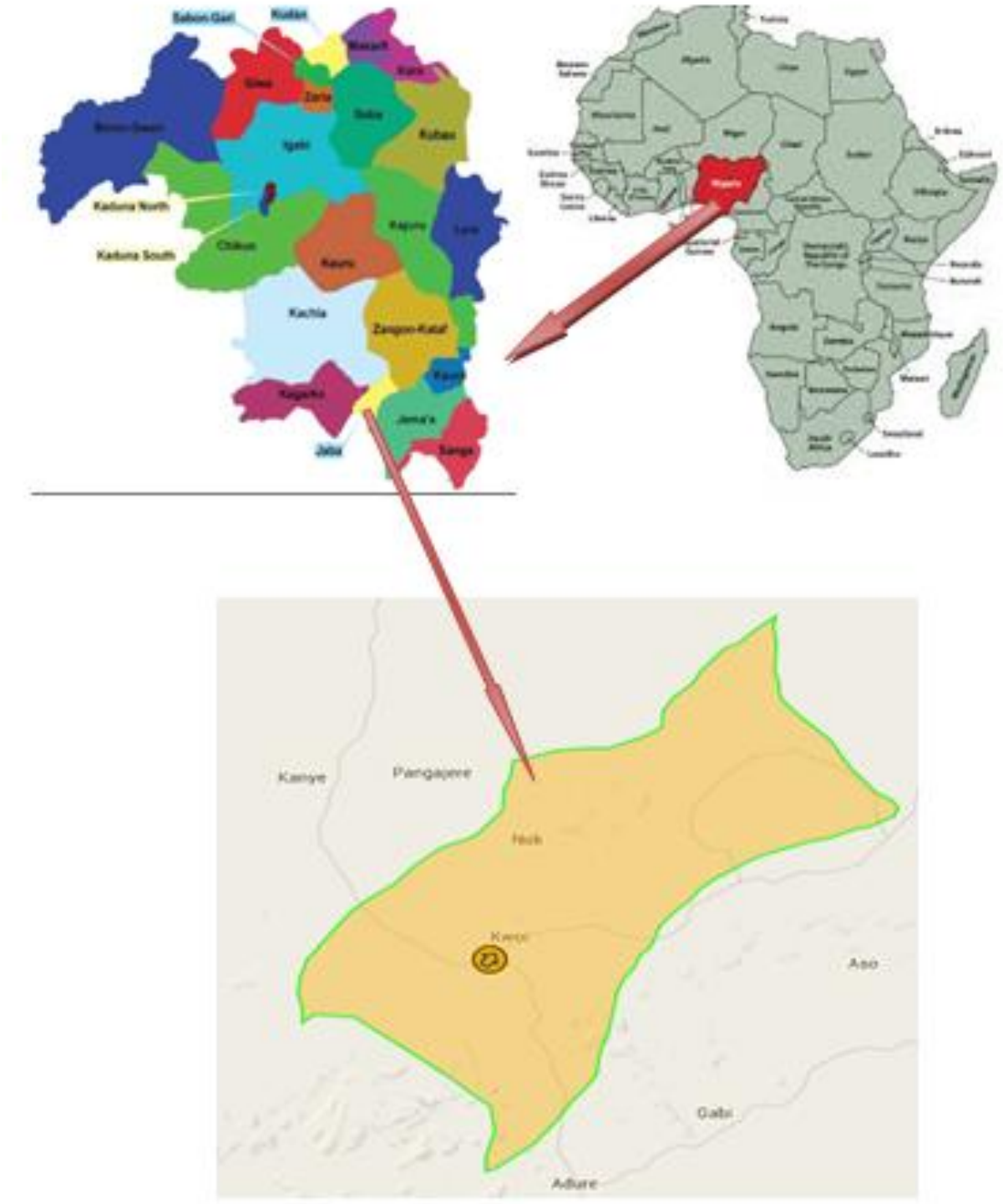


**Figure 3.1: Map of Jaba LGA in Kaduna in Kaduna State, Nigeria.**

(Map source: Maphill, 2013).

### 3.4Study Population

Primary school pupils (both female and male volunteers) in Jaba LGA were enrolled toform the studypopulation. The pupils randomly were selected from class 1-6 and of ages between 4-15years. The pupilsoften engage in water-contact activities (like swimming and fishing) and assist their parents in farm work, especially on‘Fadama’ land.

### 3.5Sample Size Determination

Using the formula by Israel (1992) for determination of sample size below, a prevalence (p) of 50% was chosen for paucity of information on the co-infection of *Salmonella* sppand urinary schistosomiasis.

Formula: $n = (Z^2pq/d^2)$

Where: n = sample size

Z = 95% confidence level with class interval (CI) of 1.96

d= allowable error of 5% (0.05)

:. q = (1- p) = 1-0.5

q = 0.5

Thus, $n = (1.96^2 x\ 0.5x0.5)/ (0.05^2) = 384.16$

However, 505 urine and 505 blood samples were collected from the pupils and examined in the laboratory.

### 3.6Collection of Samples

The following procedures were used in gathering demographic/risk factors associated with urinary schistosomiasis and salmonellosis, while urine and blood samples were collected.

### 3.6.1 Structured Questionnaires Administration

Only those pupils who submitted urine and blood samples were administered structured questionnaires. The questionnaires captured some socio-demographic and risk factors associated with urinary schistosomiasis and typhoid fever. Assistance by respective class teachers and head teachers were sought for interpretation from English language into easily understood dialect of the study area. All those who provided urine samples but disallowed collection of their blood samples were disengaged from the study. Confidentiality was applied on all data collected from them and result of laboratory tests was issued to each participated pupil. Those that had infection(s) were referred to the hospital for further medical attention.

### 3.6.2 Urine Samples Collection

The pupils who consented to participate in the study were briefed and guided on how to collect 10ml of their urine into sterile (wide-mouth) sampling bottles with screw caps between 10am -1pm (Cheesbrough, 2009).Pupils who were able to provide urine samples were simultaneously enlisted for blood sample collection.

### 3.6.3Blood SamplesCollection

A Medical Laboratory Scientist assisted in the collection of 2ml of venous blood into 5ml EDTA K-3 bottle using new sterile syringe and needle for each pupil that had submitted a urine sample.

### 3.6.4 Preparation and Transportation of Samples

The urine samples were screened away from sunlight by enclosing them in dark polythene bags to prevent hatching of *S. haematobium* eggs if present. Both sample

types from each volunteered pupil were labelled and placed in separate ice containers.The sampleswere taken for analysis in the Bacteriology/ParasitologyLaboratory ofDepartment of Microbiology, Faculty of Life Sciences, Ahmadu Bello University Zaria, Nigeria.

### 3.7Laboratory Examination of Samples

The following laboratory procedures were performed in the examinations of blood and urine samples obtained from the pupils.

### 3.7.1 Determination of Packed Cell Volume

The blood samples were brought out of the cold container and allowed to reach the room temperature in the laboratory. The packed cell volume (PCV) of each pupil was determined by the microhaematocrit centrifuge technique (HCT). Two plain capillary tubes were filled with blood sample to three-fourth their heights and sealed carefully by means of Bunsen flame to the (2 mm) red demarcation on each tube. The tubes were spun in the microhaematocrit centrifuge at relative centrifugation force(RCF)of 12,000-15,000xg for 5 minutes, after whichthe PCV wereread by correctly adjusting the red-packed-cells columns on the Haematocrit Reader and an average of the values recorded (Cheesbrough, 2009).The PCV <34% was considered anaemic, normal PCV was ≥34≤45% andPCV≥46% was considered high (Cheesbrough, 2009).

### 3.7.2 Pre-enrichment of Blood Samplesfor the Isolation of *Salmonella* spp

From each blood sample, 1ml was transferred into 5ml Selenite-F broth (Akinyemi *et al.*, 2004; Mirmomeni *et al.*, 2009), and the remaining blood sample was transferredinto 5ml Brilliant Green Bile Broth and incubated at 37$^{o}$C for 24 hrs. After the pre-enrichment period, each of the broth was sub-cultured onto prepared sterile plates of

*Salmonella-Shigella* Agar (SSA) and Xylose-Lysine-Deoxycholate (XLD) Agar and incubated at 37$^{o}$C for 24 hrs. Where growthwas not detected,the plateswerere-incubated for another 48hrs at the same temperature and periodically observed before discarding as negative culture.

### 3.7.3 Isolation of *Salmonella* spp andother Gram-negative Bacteria from Urine

The urine samples were removed from the cold container was allowed to thaw to room temperature. The samples were shakenbefore loosening the screw-caps and transferred into labelled centrifuge tubes. Where sedimentsremained in the bottle, sterile distilled water was used to rinse them into the centrifuge tube. The samples were centrifuged at 3000 rpm for 3-5 minutes.The supernatant was discarded by means of Pasteur pipette. A loopful of thesediment was inoculated on SSA and XLD agar platesand incubated at 37$^{o}$C 24 hrs. The sediment was kept forthe detection and quantification of *S. haematobium*eggs. Incubation period was increased by another 24 hrs where growths did not occur.

Blood and urine cultures were considered negative for *Salmonella* spp only after48-72hrs aerobic incubation at 37$^{o}$C. Other bacterial colonies that grew from the urine cultures were purified on the respective selective media and gram-stained to determine their Gram reactions and morphologies. The pure cultures were streaked on appropriately labelled Nutrient agar (NA) slants and incubated at 37$^{o}$C for 18-24hrs, and stored at 5$^{o}$Cfor further biochemical tests. Sub-culturing of the isolates was doneafter every two months to maintain their viability.

### 3.7.4 Detection and Quantification of *Schistosoma haematobium* eggs in Urine

The sediment obtained in the centrifuge tube (as described in unit 3.7.3 above) was tapped on the bench and mixed by gentle shaking. A Pasteur pipette was used to transfer all the sediments unto a clean, grease-free glass slide. A drop of Lugol's iodine solution was added and a cover slip was placed over the wet mount and positioned under the light microscope. The entire wet mount was screened for egg(s) of *Schistosoma haematobium*using 10x and 40x objectivesand quantitative count was taken. Where the sediments from a samplecould not be contained in a single wet mount, multiple wet mounts were made from such a sample and the egg countspooled together.

### 3.8 Biochemical Tests

The following biochemical tests were conducted to identify other Gram-negative bacteria recovered from the urine samples: sugar fermentation, gas and/or hydrogen sulphide ($H_2S$) production in Triple Sugar Iron (TSI) agar, indole production, Methyl red (MR), Voges Proskauer (VP), citrate utilisation, urease production, oxidase production, motility, lysine decarboxylation (LDC) and ornithine decarboxylation(ODC) tests (Cheesbrough, 2009).

### 3.8.1 Pattern of Sugar Fermentation and Reactions on TSI Medium

The TSI agar was prepared according to manufacturer's instruction and slanted to solidify. A cooled sterilised inoculation needle was used to pick a pure culture (from NA slant) onto appropriately labelled TSI agar slant by stabbing the butt and streaking the slant. All inoculated and uninoculated (control) TSI agar slantswere incubated at $37^oC$ for 24 hrs. Patterns of sugar fermentation, gas and/or $H_2S$ production were recorded and interpreted using standard biochemical charts.

### 3.8.2 Indole Production, Motility and Decarboxylation Reactions

Two media, Motility Indole Ornithine (MIO) and Motility Indole Lysine (MIL) were prepared according to the instructions of their manufacturers. The media were kept in upright position to solidify. Cooled sterilised inoculation needle was used to transfer pure cultures from NA slantsto inoculateappropriately labelled butts by stabbing. All the inoculated butts were incubated aerobically at 37$^{o}$C for 24 hrs. The motility of the organism was determined by evidence ofturbidity or opacity of the medium. Also, lysine or ornithine decarboxylation was indicated by light to deep purple colour formation. Both motility and decarboxylation results were first taken before indole test on MIO mediumwas performed. Three drops of Kovac's reagent were added onto the surface of the incubated medium and observed for colour change. Red colour formation indicated an indole production, but yellow colour meant negative indole test.

### 3.8.3 Methyl red (MR) and Voges Proskauer (VP) Tests

The MR-VP medium was prepared following instruction of the manufacturer. A labelled tube containing 8ml of themedium was inoculated with the test organismfrom NA slant and incubated at 37$^{o}$C for 24 hrs. After that time period, the medium was divided into two (by pouring about half of the volume into a labelled sterile tube-2). The remaining medium was labelled tube-1 and re-incubated for another 24 hrs. Tube-2 was used for MR test by adding 3 drops of Methyl red reagent followed by gentle shaking to mix. A stable red colour in the medium was taken as positive MR test; but where the colour was unstable or disappeared immediately, it was taken as a negative result. After the re-incubation of theformer tube(i.e., tube-2), itwas used for VP test. Into the medium, 10 drops of α-naphthol (Barritt's A) were added and shaken vigorously to mix. Then, 5 drops of 40% KOH (Barritt's B) were added and well shaken. The medium

wasleft to react for 20 mins after which red colour formation was taken as positive VP test; otherwise it was a negative result.

### 3.8.4 Citrate Utilisation Test

This was conducted by aseptically stabbing the butt and streaking the slants of prepared Simmons Citrate Medium with pure culture of the test organism. Inoculated tubes were incubated at 37$^{o}$C for 24 hrs. A change in colour of the medium from green to blue indicated the utilisation of citrate as thesole carbon source. Unchanged tubes were taken as negative after 48 hrs incubation at 37$^{o}$C.

### 3.8.5 Urease Test

Urease Agar Base was prepared according to manufacturer's specification. Then 5mls of sterile 40% urea was added and mixed. The mixture was dispensed into sterile test tubes and slanted to solidify. After labelling the tube, the test organismwas aseptically stabbed into the butt and streaked onto the slant. Inoculated tubeswere incubated at 37$^{o}$C for 24 hrsto observe production of urease.The change of colour in the medium from orange-yellow to pink was observed. However, after 7 days incubation at the same conditions, unchanged tubes (that remained orange-yellow) were taken as negative (Vlab, 2011).

### 3.8.6 Oxidase Test

Fresh oxidase reagent was prepared following the instruction of the manufacturer. Whattman filter (No. 1) paper was placed on the workingbench and the test isolate was smeared. A drop of the prepared oxidase reagent was placed to cover the smear. Positive oxidase test was recorded as a formation of a deep purple colour within 8-10 seconds.

Absence of this colour or its appearance later than 10 seconds was taken as negative result.

### 3.9Data Analyses

Data collected on some socio-demographic and risk factors of urinary schistosomiasis and *Salmonella* sppinfections were subjected to ANOVA, Chi Square ($x^2$), Likelihood ratio (LR) and Odd Ration (OR) analyses with the IBM SPSS Statistics Version 21 at $P=0.05$. Results were summarized in tables, charts and plates.

# CHAPTER FOUR

## 4.0 RESULTS

Out of 505 urine samples of pupils examined, *Schistosoma haematobium* ova were detected in 62 of the samples with a prevalence of 12.3 % (Figure4.1). The eggs of this parasite wereyellow-brown in colour, oval in shape with terminal spines;someof the eggs were shorter but majority were slender (Plate I).

Gender as a factor in the occurrence of urinary schistosomiasis was subjected to a Chi Square test with statistically significant difference obtained ($\chi^2$ = 4.926; df = 1; P= 0.026, Odd Ratio (OR) =0.541)as indicated in Table 4.1. There was a remarkable higher occurrence of urinary schistosomiasis among the female (15.5%) than in the male pupils (9.1%). Hence, female pupils were about twice more infected than the male pupils(Relative Risk, RR =1.716). Also, the female pupils had a higher range of egg count of 0-204/10ml of urine than the male pupils who had a range of 0-78eggs/10ml. Mean intensity in the female pupils (4.18±1.202 eggs/10ml) was significantly higher (ANOVA (F) = 5.227; df = 1; P = 0.023) compared to 1.22±0.500eggs/10ml in the male (Table 4.1).

There was statistically significant difference in age prevalence of urinary schistosomiasis among the pupils ($\chi^2$= 37.700; df = 11;P = 0.000). No infection was detected in pupils within age-group of 4-6 years compared to those of 13-15 years who had the highest prevalence of 27.9% and the highest intensity of 9.97±3.915eggs/10ml of urine. However, the prevalence and intensity of urinary schistosomiasis increased with increase in age of the pupils (Table 4.2).

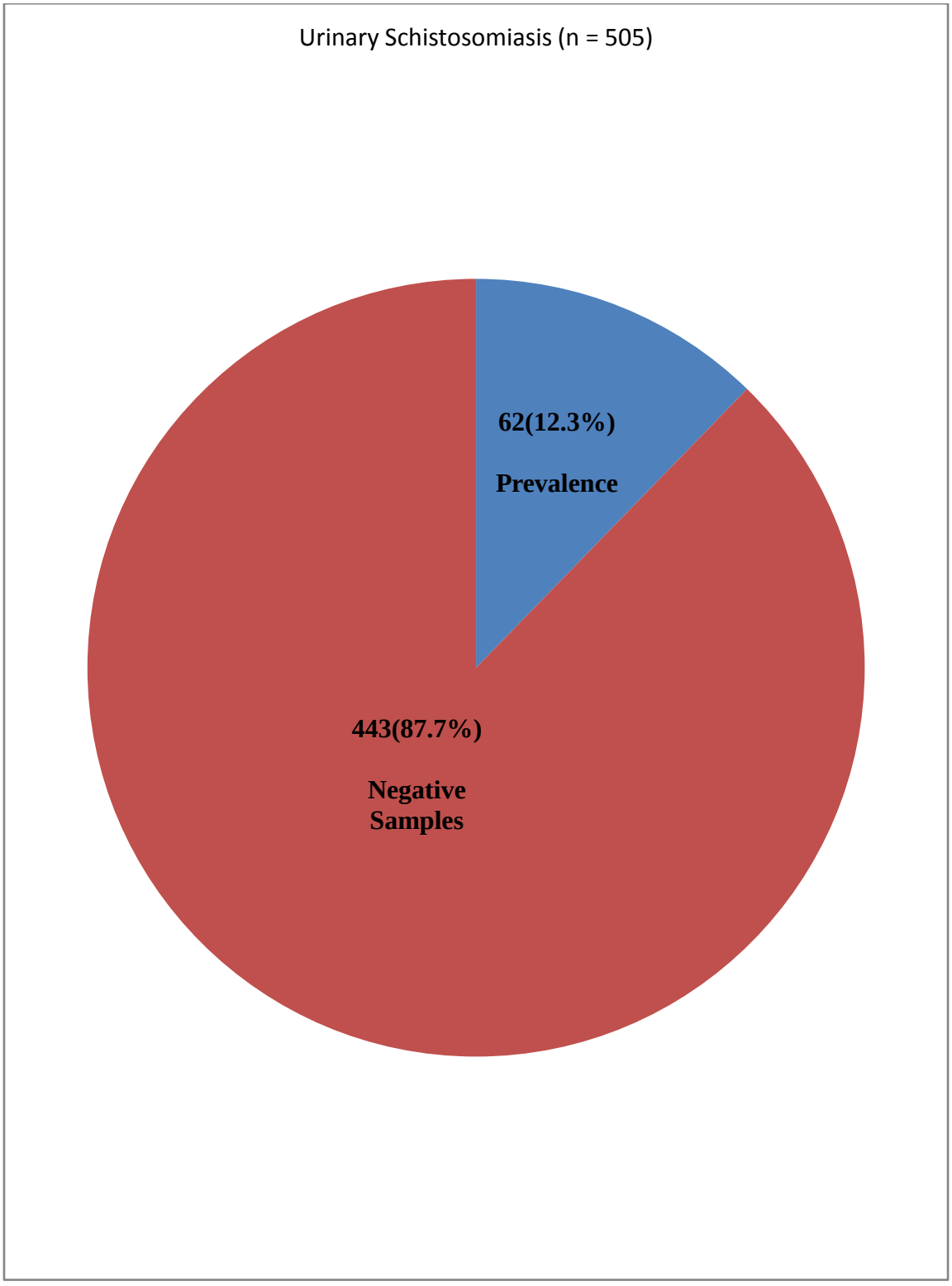


**Figure 4.1: Prevalence of Urinary Schistosomiasis among Pupils of Jaba LGA, Kaduna State, Nigeria.**

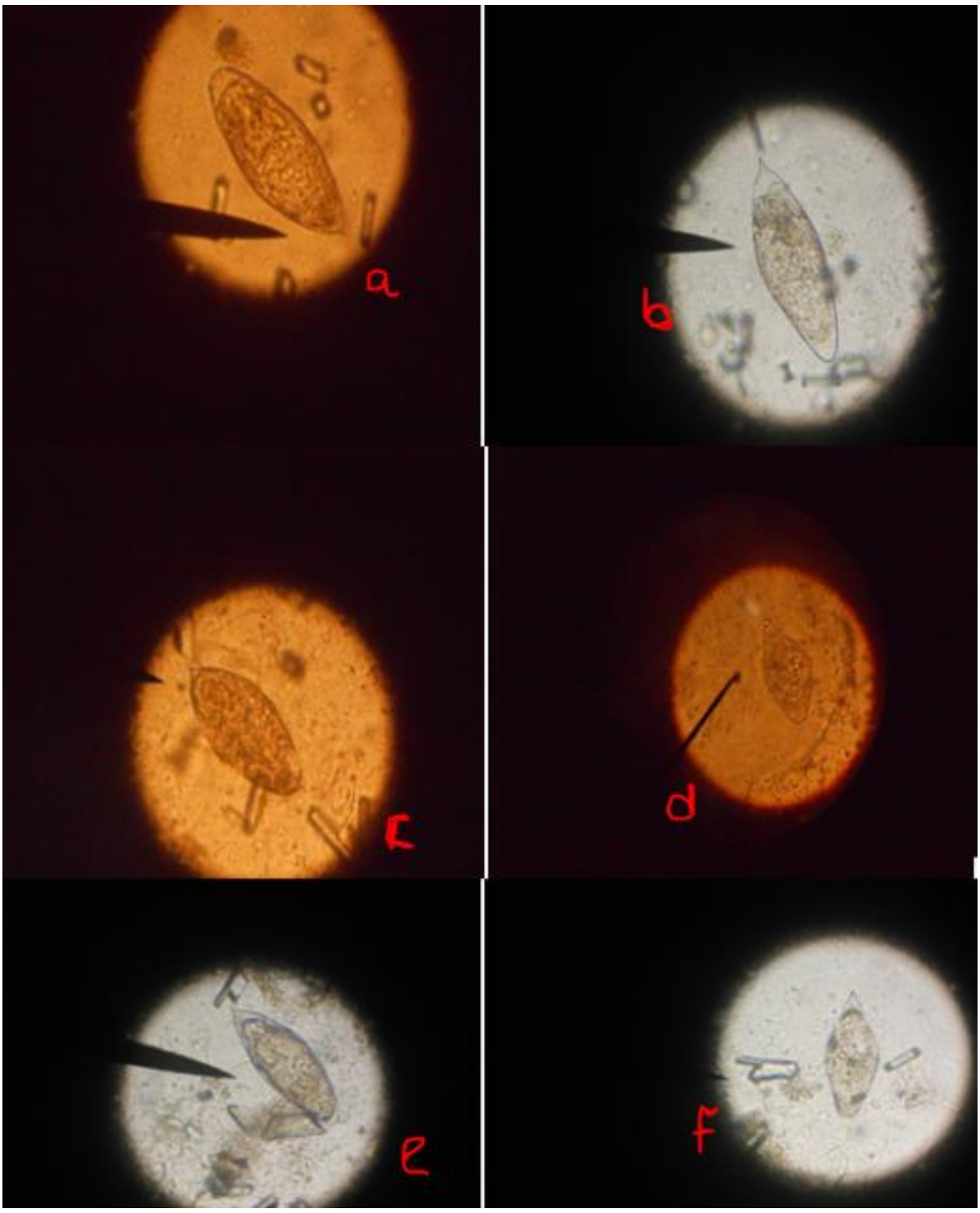


**Plate I: Various appearances of *S. haematobium* ova in urine samples of pupils from Jaba LGA, Kaduna State, Nigeria.**

(a, c, d, in wet mounts stained with Lugol's iodine; b, e, f, without Logol's iodine)

**Table 4.1: Gender-related Prevalence and Intensity of Urinary Schistosomiasis among Pupils in Jaba LGA, Kaduna State, Nigeria**

| Gender | No. of Samples Examined | *Number Positive (%) | Range of Egg Count | **MeanIntensity/10ml of Urine±SEM |
|---|---|---|---|---|
| Female | 251 | 39 (15.5) | 0-204 | 4.18±1.202 |
| Male | 254 | 23 (9.1) | 0-78 | 1.22±0.500 |
| **Total** | **505** | **62 (12.3)** | **0-204** | 2.69±0.651 |

*$\chi^2$ = 4.926; df = 1; P = 0.026, LR = 4.973; df = 1; P = 0.026, OR = 0.541

**ANOVA (F) = 5.227; df = 1; P = 0.023

Key: SEM = Standard Error of Mean

**Table 4.2: Age Prevalence and Intensity of Urinary Schistosomiasis among Pupils in Jaba LGA, Kaduna State, Nigeria**

| Age-Group (Years) | No. of Samples Examined | *Number Positive (%) | Range of Egg Count | **MeanIntensity/10ml of Urine±SEM |
|---|---|---|---|---|
| 4-6 | 31 | 0 (0.0) | 0 | 0.00±0.00 |
| 7-9 | 185 | 16 (8.6) | 0-130 | 1.59±0.889 |
| 10-12 | 228 | 29 (12.7) | 0-78 | 2.01±0.627 |
| 13-15 | 61 | 17 (27.9) | 0-204 | 9.97±3.915 |
| **Total** | **505** | **62(12.3)** | **0-204** | **2.69±0.651** |

*$\chi 2 = 20.411$; df = 3; P = 0.000, LR = 21.302; df = 3; P = 0.000

**ANOVA (F) = 6.079; df = 3; P = 0.000

Key: SEM = Standard Error of Mean

The prevalence of urinary schistosomiasis according to sampling locations in Jaba LGA by statistics was significantly different($\chi^2$ = 38.599; df = 9; P = 0.000).The highest occurrence of the disease was found among pupils from Bitaro (23.2%) followed by Ankun (22.2%) and Kwoi (20.3%), whereas no occurrences were recorded among pupils from Nok (0.0%) and Sambang (0.0%) as shown in Table 4.3.

The mean intensity of *Schistosoma haematobium* eggs in urine samples of pupils from the 10 different sampling locations were analysed by means of ANOVA. Although no statistical significant difference was obtained (F = 1.419; df = 9; P = 0.177), the highest intensities were 6.77 and 4.88among pupils from the Central Area and Bitaro respectively(Table 4.3).

The level of urinary schistosomiasis among pupils versus sampling locations showed statistically significance difference as shown in Table 4.4 ($\chi^2$= 50.094, df = 18, *P* = 0.000). This was categorized as 'light' and 'heavy' infections, with egg counts of <50eggs/10ml and ≥50eggs/10ml of urine respectively. Light infections was found mostly among pupils from Bitaro (44.2%), followed by those from Kwoi (21.2%) and Ankun (9.6%). Heavy infections were most found among pupils from the Central Area (40.0%), followed by those from Bitaro (30.0%). However, light infection was absent among pupils from Nok and Sambang, while heavy infection was not found in pupils from Chori, Dura, Gora, Nok and Sambang.

In Table 4.5, there was statistical significant difference in the age-related distribution of level of urinary schistosomiasis among the pupils in Jaba LGA ($\chi^2$ = 22.204; df = 6; P = 0.001, LR = 22.209 df = 6; P = 0.001). Both heavy and light infections occurred mostly in pupils within age-group 13-15 years, but absent in those of 4-6 years of age. However, occurrences of light and heavy infections increased with the age of the pupils.

**Table 4.3: Prevalence and Intensity of Urinary Schistosomiasis according to Sampling Locations in Jaba, Kaduna State, Nigeria**

| Sampling Location | No. of Samples examined | *Number Positive (%) | **Intensity (Mean of Eggs/10ml of urine ± SEM) |
|---|---|---|---|
| Ankun | 27 | 6 (22.2) | 3.30±2.301 |
| Bitaro | 112 | 26(23.2) | 4.85±2.018 |
| Central Area | 57 | 7(12.3) | 6.77±3.335 |
| Chori | 30 | 1(3.3) | 0.10±0.100 |
| Dura | 21 | 1(4.8) | 0.29±0.286 |
| Gora | 26 | 4(15.4) | 0.73±0.358 |
| Kwoi | 59 | 12(20.3) | 2.73±1.451 |
| Nok | 33 | 0(0.0) | 0.00±0.000 |
| Sambang | 78 | 0(0.0) | 0.00±0.000 |
| Yadi-Pyok | 62 | 5(8.1) | 2.47±1.443 |

* $\chi 2 = 38.599$; df = 9; P = 0.000, LR = 50.200; df = 9; P = 0.000

**ANOVA Value (F) = 1.419; df = 9; P = 0.177

Key: SEM = Standard Error of Mean

**Table 4.4: Distribution of Level of Urinary Schistosomiasis across Sampling Locations in Jaba LGA, Kaduna State, Nigeria**

| Level of Infection | Samples Size | Ankun | Bitaro | Central Area | Chori | Dura | Gora | Kwoi | Nok | Sambang | Yadi-Pyok |
|---|---|---|---|---|---|---|---|---|---|---|---|
| None | 443 | 21 (4.7) | 86 (19.4) | 50 (11.3) | 29 (6.5) | 20 (4.5) | 22 (5.0) | 47 (10.6) | 33 (7.4) | 78 (17.6) | 57 (12.9) |
| Light | 52 | 5 (9.6) | 23 (44.2) | 3 (5.8) | 1 (1.9) | 1 (1.9) | 4 (7.7) | 11 (21.2) | 0 (0.0) | 0 (0.0) | 4 (7.7) |
| Heavy | 10 | 1 (10.0) | 3 (30.0) | 4 (40.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (10.0) | 0 (0.0) | 0 (0.0) | 1 (10.0) |
| **Total** | **505** | **27 (5.3)** | **112 (22.2)** | **57 (11.3)** | **30 (5.9)** | **21 (4.2)** | **26 (5.1)** | **59 (11.7)** | **33 6.5)** | **78 (15.4)** | **62 (12.3)** |

$\chi^2 = 50.094$, df = 18, P = 0.000, LR = 59.532; df = 18; P = 0.000

**Table 4.5: Age-related Distribution of Level of Urinary Schistosomiasis among Pupils in Jaba LGA, Kaduna State, Nigeria**

| Age-Group (Years) | No. of Samples Examined | Light Infection No. (%) | Heavy Infection No. (%) |
|---|---|---|---|
| 4-6 | 31 | 0 (0.0) | 0 (0.0) |
| 7-9 | 185 | 14 (7.6) | 2 (1.1) |
| 10-12 | 228 | 25 (11.0) | 4 (1.8) |
| 13-15 | 61 | 13 (21.3) | 4 (6.6) |
| **Total** | **505** | **52(10.3)** | **10(2.0)** |

$\chi^2 = 22.204$; df = 6; P = 0.001, LR = 22.209 df = 6; P = 0.001

There was no occurrence of *Salmonella* spp from the blood samples of pupils in Jaba LGA (0.0%). Also, no *Salmonella* spp was isolated in the urine samples of the pupils (0.0%). Similarly, there was no co-infection between *Schistosoma haematobium* and *Salmonella* spp (0.0%) in this study as shown in Table 4.6.

The prevalence of anaemia among pupils in Jaba LGA was found to be 8.1%, while 37.6% of the pupils had normal PCV. The remaining study population (54.3%) had abnormally high PCV (Figure 4.2).

In Table 4.7, there was a statistically significant association between urinary schistosomiasis and anaemia among the pupils ($\chi^2$ = 11.870; df = 2;P = 0.003). Though anaemia was recorded both among those with urinary schistosomiasis and uninfected pupils, a higher occurrence of the anaemia (17.7%) was observed in pupils infected with urinary schistosomiasis than those who were not infected (6.8%).

Heavy infections with urinary schistosomiasis among the pupils, with a statistical significant difference ($\chi^2$= 12.807; df = 4; P = 0.012) led to higher occurrence of anaemia (20.0%) than light infections which caused 17.2% of the anaemia (Table 4.8).

Though female pupils had higher proportions of light and heavy infections than the male pupils in Jaba LGA, the difference was not statistically significant ($\chi^2$ = 5.166; df = 2; P = 0.076) as shown in Table 4.9.

**Table 4.6: Prevalence of *Salmonella* species and its Co-infections with Urinary Schistosomiasis in Jaba LGA**

| Sample Type | Number of Samples Examined | *Salmonella* spp Number Positive (%) | *S. haematobium* Number Positive (%) | Co-infection Number Positive (%) |
|---|---|---|---|---|
| Blood | 505 | 0(0.0) | ** | ** |
| Urine | 505 | 0(0.0) | 62 (12.3) | 0(0.0) |

**Not applicable

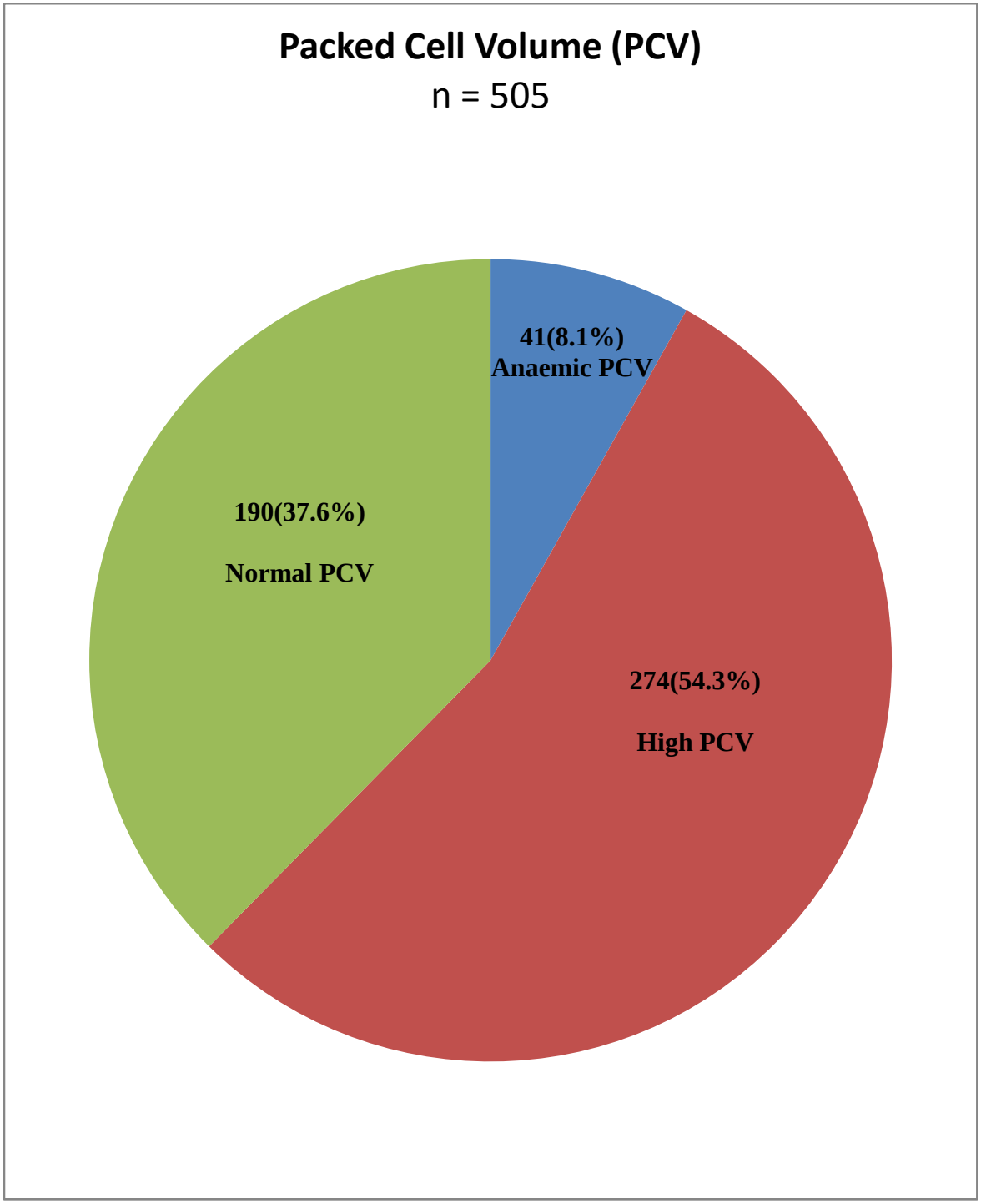


**Figure 4.2: Prevalence of Anaemia among the Pupils in Jaba LGA, Kaduna State, Nigeria.**

**Keys:** Anaemic PCV was <34%,

Normal PCV was ≥34≤45%,

High was ≥46%

**Table 4.7: Relationship between Urinary Schistosomiasis and Anaemia among Pupils in Jaba LGA, Kaduna State, Nigeria**

| Status of Urinary Schistosomiasis | No. of Samples Examined | Anaemic PCV No. Positive (%) | Normal PCV No. Positive (%) | High PCV No. Positive (%) |
|---|---|---|---|---|
| Not Infected | 443 | 30 (6.8) | 163 (36.8) | 250 (56.4) |
| Infected | 62 | 11 (17.7) | 27 (43.5) | 24 (38.7) |
| **Total** | **505** | **41 (8.1)** | **190 (37.6)** | **274 (54.3)** |

$\chi^2 = 11.870$; df = 2; P = 0.003, LR = 10.401; df = 2; P = 0.006

**Table 4.8: Effect of the Level of Urinary Schistosomiasis on Pupils' Packed Cell Volume in Jaba LGA, Kaduna State, Nigeria**

| Level of Infection | No. of Samples Examined | Anaemic PCV No. Positive (%) | Normal PCV No. Positive (%) | High PCV No. Positive (%) |
|---|---|---|---|---|
| None | 443 | 30 (6.8) | 163 (36.8) | 250 (56.4) |
| Light | 52 | 9 (17.3) | 24 (46.2) | 19 (36.5) |
| Heavy | 10 | 2 (20.0) | 3 (30.0) | 5 (50.0) |
| **Total** | **505** | **41(8.1)** | **190 (37.6)** | **274 (54.3)** |

$\chi^2 = 12.807$; df = 4; P = 0.012, LR = 11.353; df = 4; P = 0.023

**Table 4.9: Relationship of Level of Urinary Schistosomiasis and Gender of Pupils in Jaba, Kaduna State, Nigeria**

| Level of Infection | Numberof Samples Examined | Female Number Positive (%) | Male NumberPositive (%) |
|---|---|---|---|
| None | 443 | 212 (47.9) | 231 (52.1) |
| Light | 52 | 32 (61.5) | 20 (38.5) |
| Heavy | 10 | 7 (70.0) | 3 (30.0) |

$\chi^2$= 5.166; df = 2; P = 0.076, LR = 5.237; df = 0.073

The level of the urinary schistosomiasis did not influence the onset of (visible) haematuria among the pupils in this study ($\chi^2$ = 3.105; df = 2;P = 0.212). All the pupils with heavy infections did not present with haematuria. Generally, 4.5% of the pupils presented with haematuria but no eggs of *Schistosoma haematobium* were recovered. However, 9.6% of the pupils with light infections presented with haematuria (Table 4.10).

As shown in Table 4.11, five risk factors of urinary schistosomiasis among the pupils were considered in this study. There was a statistically significant association between urinary schistosomiasis and 'Fadama' farming ($\chi^2$= 14.300; df = 1;P = 0.000, *OR = 2.789*). All the pupils that made up the study population were unaware of schistosomiasis. Pupils that wash their clothes in rivers and streams(OR =1.428) had higher infections (14.7%) than those who wash their clothes at home (10.8%). Similarly, pupils who claimed not to indulge in swimming and/or fishing in streams/rivers had more cases of urinary schistosomiasis than those who did.

Appearances of some of the rivers in Jaba LGA of Kaduna State in Nigeria are shown in Plate II. Most of the rivers are surrounded by immediate wet fields locally referred as 'Fadama' for rice, cocoyam and sugarcane farming. The rivers are used for swimming, fishing, irrigation and washing by pupils and the members of the communities.

The level of formal education of the pupils' parents had no statistical relationship with acquisition of urinary schistosomiasis by their children/wards (P > 0.05). However, lowest detection of *Schistosoma haematobium* eggs was observed in pupils whose parents did not acquire any formal education. Occurrence of the infection among the pupils increased as fathers' level of formal education increased (Table 4.12).

**Table 4.10: Effect of Level of Urinary Schistosomiasis on Occurrence of Haematuria among Pupils in Jaba LGA, Kaduna State, Nigeria**

| **Level of infection** | **No. of Samples Examined** | **Absence of Haematuria No. Positive (%)** | **Presence of Haematuria No. Positive (%)** |
|---|---|---|---|
| None | 443 | 423 (95.5) | 20 (4.5) |
| Light | 52 | 47 (90.4) | 5 (9.6) |
| Heavy | 10 | 10 (100.0) | 0 (0.0) |
| **Total** | **505** | **480 (95.0)** | **25 (5.0)** |

$\chi^2 = 3.105$; df = 2; P = 0.212, LR = 3.108; df = 2; P = 0.211

**Table 4.11: Risk Factors associated with Urinary Schistosomiasis among Primary School Pupils in Jaba LGA, Kaduna State, Nigeria**

| Risk Factor | Response Category | No. of Samples Examined | Positive No. (%) | Negative No. (%) |
|---|---|---|---|---|
| Awareness of Schistosomiasis[#] | Unaware | 505 | 62 (12.3) | 443 (87.7) |
| | Aware | 0 | 0 (0.0) | 0(0.0) |
| 'Fadama' Farming[*] | Yes | 129 | 28 (21.7) | 101 (78.3) |
| | No | 376 | 34 (9.0) | 342 (91.0) |
| Fishing in River/Stream[**] | Yes | 109 | 10 (9.2) | 99 (90.8) |
| | No | 396 | 52 (13.1) | 344 (86.9) |
| Swimming in River/Stream[+] | Yes | 327 | 35 (10.7) | 292 (89.3) |
| | No | 178 | 27 (15.2) | 151 (84.8) |
| Place of Laundering[@] | River/Stream | 190 | 28 (14.7) | 162 (85.3) |
| | Home | 315 | 34 (10.8) | 281 (89.2) |

[#]No computed statistics because unawareness was a constant.

* $\chi^2$= 14.300; df = 1; P = 0.000, LR = 12.915; df = 1; P = 0.000, OR =2.789

** $\chi^2$ = 1.243; df =1 ;P = 0.265, LR = 1.319; df = 1; P = 0.251,OR = 0.668

+ $\chi^2$= 2.134; df =1 ;P = 0.144, LR = 2.080; df = 1; P = 0.149, OR = 0.670

@ $\chi^2$= 1.711; df = 1; P = 0.191, LR = 1.679; df = 1; P = 0.195, OR = 1.428


River Dhugk Gira


River Langk Wada

**Plate II: River Dhugk Gira and River Langk Wada, the most popular rivers in Bitaro, Jaba LGA, Kaduna State, Nigeria**
(Used for swimming, fishing and washing by pupils and members of the community)

**Table 4.12: Association of Parental Level of Formal Education and Urinary Schistosomiasis Status of Pupils in Jaba LGA, Kaduna State, Nigeria**

| Parent Category | Level of Formal Education | Number of Samples Examined | NumberPositive (%) | Number Positive (%) |
|---|---|---|---|---|
| **Mother*** | None | 58 | 2 (3.4) | 56 (96.6) |
| | Primary | 94 | 13 (13.8) | 81 (86.2) |
| | Secondary | 302 | 43 (14.2) | 259 (85.8) |
| | Tertiary | 51 | 4 (7.8) | 47 (92.2) |
| | **Total** | **505** | **62 (12.3)** | **443 (87.7)** |
| **Father**** | None | 59 | 4 (6.8) | 55 (93.2) |
| | Primary | 63 | 6 (9.5) | 57 (90.5) |
| | Secondary | 299 | 40 (13.4) | 259 (86.6) |
| | Tertiary | 84 | 12 (14.3) | 72 (85.7) |
| | **Total** | **505** | **62 (12.3)** | **443 (87.7)** |

* $\chi^2$= 6.418; df = 3; P = 0.093, LR = 7.947; df = 3; P = 0.047

** $\chi^2$ = 2.750; df = 3; P = 0.432, LR = 3.041; df = 3; P = 0.385

From data obtained of signs and symptoms using questionnaires (Appendix I), only painful micturition ($\chi^2$ = 5.135; df = 1; P = 0.023, OR = 2.586) and cloudy-brown/red urine ($\chi^2$ =20.604; df = 4; P = 0.000) had statistical significant associations with urinary schistosomiasis. There were higher occurrences of *Schistosoma haematobium* eggs in pupils' urine samples with haematuria ($\chi^2$ =1.457; df = 1; P = 0.227, OR = 1.855) and in those fever ($\chi^2$ = 1.714; df = 1; P = 0.190, OR = 2.113) as shown in Table 4.13.

As shown in Table 4.14, out of 505 urine samples cultured, 73 were culture-positive (14.5%). Seven different bacterial uropathogens were identified (Appendix II and III). The most occurring bacteria were *Citrobacter* spp (6.7%) and *Klebsiella* spp(3.4%). But *Providencia* spp (0.2%) and *Serratia marcescens*(0.2%) were the least occurring bacteria. One ovum *of Enterobius vermicularis* was identified from urine sample of a female pupil. Three of these bacterial uropathogens had very low co-infections with urinary schistosomiasis, namely,*Citrobacter* spp (1.0%), *Escherichia coli* (0.2%) and *Klebsiella* spp (0.2%).

**Table 4.13: Sign/Symptoms Associated with Urinary Schistosomiasis among the Pupils, Jaba LGA, Kaduna State, Nigeria**

| Symptom | Category | No. of Samples Examined | No. (%) Positive | No. (%) Negative |
|---|---|---|---|---|
| Haematuria* | No | 480 | 57 (11.9) | 423 (88.1) |
| | Yes | 25 | 5 (20.0) | 20 (80.0) |
| Painful micturition** | No | 473 | 54 (11.4) | 419 (88.6) |
| | Yes | 32 | 8 (25.0) | 24 (75.0) |
| Frequent micturition# | No | 496 | 61 (12.3) | 435 (87.7) |
| | Yes | 9 | 1 (11.1) | 8 (88.9) |
| Abdominal pain[a] | No | 496 | 61 (12.3) | 435 (87.7) |
| | Yes | 9 | 1 (11.1) | 8 (88.9) |
| Fever[b] | No | 487 | 58 (11.9) | 429 (88.1) |
| | Yes | 18 | 4 (22.2) | 14 (77/8) |
| Urine colour[c] | Brown and cloudy | 2 | 1 (50.0) | 1 (50.0) |
| | Cloudy | 39 | 5 (12.8) | 34 (87.2) |
| | Milky-white | 91 | 9 (9.9) | 82 (90.1) |
| | Red and cloudy | 92 | 23 (25.0) | 69 (75.0) |
| | Yellow-orange | 281 | 24 (8.5) | 257 (91.5) |

* $\chi^2$ =1.457; df = 1; P = 0.227, LR = 1.266; df = 1; P = 0.260, OR = 1.855
** $\chi^2$ = 5.135; df = 1; P = 0.023, LR 3.951; df = 1; P = 0.041, OR = 2.586
# $\chi^2$ = 0.012; df = 1; P = 0.914, LR = 0.012, df = 1; P = 0.913, OR = 0.891
[a] $\chi^2$ = 0.012; df = 1; P = 0.914, LR = 0.012, df = 1; P = 0.913, OR = 0.891
[b] $\chi^2$ = 1.714; df = 1; P = 0.190, LR = 1.439; df = 1; P = 0.230, OR = 2.113
[c] $\chi^2$ =20.604; df = 4; P = 0.000, LR = 17.315; df = 4; P = 0.02, OR =inapplicable

**Table 4.14: Other Bacteria and Parasites and their Co-infections with *Schistosomahaematobium* among Pupils in Jaba LGA of Kaduna State, Nigeria**

| Isolated Organism | Prevalence No. (%) | *Co-infection with *S. haematobium* No. (%) |
|---|---|---|
| *Acinetobacter* spp | 10 (2.0) | 0(0.0) |
| *Citrobacter* spp | 34(6.7) | 5(1.0) |
| *E. coli* | 8(1.6) | 1(0.2) |
| *Klebsiella* spp | 17(3.4) | 1(0.2) |
| *Providencia* spp | 1(0.2) | 0(0.0) |
| *Pseudomonas aeruginosa* | 2(0.4) | 0(0.0) |
| *Serratia marcescens* | 1(0.2) | 0(0.0) |
| Negative cultures | 432 (85.5) | 55 (88.7) |
| *Enterobius vermicularis* ovum | 1(0.2) | 0(0.0) |
| **Total** | **505 (100.0)** | **62(100.0)** |

n=505

# CHAPTER FIVE

## 5.0 DISCUSSION

In this study, the presence of urinary schistosomiasis was established by the detection of terminal spine eggs in urine samples of pupils.The prevalence of 12.3% for urinary schistosomiasis among the pupilsin Jaba LGA, Kaduna State, agreed with the prevalence of 12.3% reported among school children in Lere LGA also in Kaduna State (Luka *et al.*, 2001).

However, the prevalence of urinary schistosomiasis in this study was much lower than many other findings from differentparts of Nigeria. The 12.3% prevalence in this study was lower than the31.3% prevalence reported by Ifeanyi *et al.* (2009) in Abuja, Nigeria. Other higher prevalence reports from Nigeria include 79.4% among children of Ezza-North LGA of Ebonyi (Uneke *et al.*, 2009), 41.5% in Benue (Houmsou *et al.*, 2012), 78.4% in Lagos (Oluwasogo and Fagbemi 2013), 36.0% in Birni-Gwari LGA of KadunaState (Alhassan *et al.*, 2013), 34.1% in Enugu (Ossai *et al.*, 2014), 48.7% in Borno (Balla *et al.*, 2015), and 19.5% in Zaria (Omenesa *et al.*, 2015). Compared to these high-prevalence areas, Jaba LGA had a lower burden of urinary schistosomiasis.

JabaLGA has few fast-flowing streams and rivers that may not provide adequate breeding zone for cercariae. Some of the streams and rivers are seasonal; they dry up during the dry season coinciding with when children most indulgein water-contact activities. This helped to limit their risk of coming in contact with schistosome cercariae. Also, many of the streams andrivers in the area are used for washing of clothes. Detergents and soaps have adverse effects on cercariae and snails.Hence, these agents help in shortening the lifecycle and propagation of schistosome cercariae as outlined by Grimes *et al.* (2014).

Also, this study has shown a low prevalence of urinary schistosomiasis compared to 15.2% prevalence reported in Mali by Dabo *et al.* (2011). A prevalence of 20.7% was found in a retrospective study in Kumasi by Tay *et al.* (2011), and 30.7% by Tetteh-Quarcoo *et al.* (2013) all in Ghana. A considerably higher prevalence of 35.9% has been reported by Geleta *et al.* (2015) in Ethiopia, and 42% among primary school children by in South Africa (Samie *et al.*, 2010).

Although a relatively low prevalence was obtained in this study, it is still higher than reported 4.5% prevalence in Abini community of Cross River, Nigeria (Ingang-Etoh *et al.*, 2009). The prevalence of urinary schistosomiasis in this study was also higher than reported 6.3% prevalence among children of Ngbo-West LGA in Ebonyi, Nigeria (Uneke *et al.*, 2009), and 8.3% in Kano, Nigeria (Dawaki *et al.*, 2015).

There was absence of*Salmonella* spp in this study population based on urine and blood examination. Pegues and Miller (2009) and Singh *et al.* (2011) reported that the isolation of *Salmonella* from urine was rare, even in the endemic areas. The *Salmonella* spp are not continuously excreted, but may be isolated from urine of 25% of typhoid patients in the third week of infection, especially among those co-infected with urinary schistosomiasis (Cheesbrough, 2006).

Reasonsfor theabsence of*Salmonella* sppin this study are not far-fetched: firstly, the study population was made up of apparently healthy pupils. Secondly, only 18(3.6%) and 9(1.8%) of this population reported the presence of fever and abdominal pain respectively, yetthere was nooccurrenceof *Salmonella* spp in their blood and urine samples. Typhoid fever presents with discomforting symptomsand a capacity to immobilize a sufferer. Hence, infected pupils might nothave been in school during the study.

The absenceof *Salmonella* spp in blood samples in this study agreed with the research findings of Akinyemi *et al.* (2004) who also did not find *Salmonella* spp in blood samples. However, it contrasted the report of Singh *et al.* (2011) who obtained 15.0% prevalence for *Salmonella* spp from urine samples. Also, Abdullahi *et al.* (2015) showed that 104 hospitalized patients had *Salmonella* spp out of 500 that presentedwith typical symptoms of *Salmonella* infections: (vomiting and/or diarrhoea, headache, abdominal pain, body pain, dyspnea, weight lost, constipation and anaemia).

Immunological factors can affectapathogenic organism within the blood as well the inhibitory effect of ethylene-diamine-tetraacetic acid in the EDTA K-3 sampling bottles. A combination of these factorscan affect recovery of isolates from blood samples (Mikoleit, 2010). The inhibitory effects of EDTA had been studied by Root *et al.* (1988), Stevens *et al.* (1991), Chudzik *et al.* (2007), Hinton and Ingram (2010).

There was no co-infection of *Schistosoma haematobium* and *Salmonella* spp in this study. This finding revealed that these co-infections are not always common in urinary schistosomiasis endemic areas,though it had been assertedby Melhem and LoVerde (1984) and Barnhill *et al.* (2011) from their studies that *Schistosoma* and *Salmonella*exhibit synergistic interactions. The absence ofthese co-infections disagreed with the finding of Igwe and Agbo (2014) for co-occurrence of *Salmonella* spp and *Schistosoma* spp in endemic areas.

The significantly higher prevalence of urinary schistosomiasis amongst the female pupils compared to that ofthe male is among the few reports of higher occurrence of schistosomiasis associated with female gender. Our finding is similar to the findingof Oluwasogo and Fagbemi (2013)in which female subjects (60.9%) were more infected than the male (39.2%).Butthis observed phenomenon disagreedwith many other

findings which had suggested higher risks onthe male (Luka *et al.*, 201; Uneke *et al.*, 2009; Ifeanyi *et al.*, 2009; Bigwan *et al.*, 2013; Alhassan *et al.*, 2013; Ossai *et al.*, 2014; Omenesa *et al.*, 2015). Most of those studies that had implicated the male gender with higher risk of *Schistosoma* infections had been conducted in locations where femalesare restricted from open swimming activity. In Jaba LGA, both the female and male subjectswere nothindered bytraditional or religious restrictions from participating in open swimming activity. However, there was an unequal exposure risks between the genders; the female subjects participated more in rice farming, other 'Fadama' activities and washing of clothes in open streams and rivers.

Aside the obviously high prevalence of urinary schistosomiasis in the female pupils, this study revealed a higher percentage of heavy infections among the femalethan among the male. This indicated possible repeated re-infections among the female pupils who come more in contact with unsafe water bodies during farm work in 'Fadama' land, fetching of water or washing of clothes in such water bodies, or domestic use of water from other unsafe sources.

An inclusion of behavioural difference between the genders could be used to explain reasons for the high level of urinary schistosomiasis among the female pupils. Schistosomes are attracted to skin lipids (Sakanari and Mckerrow, 2010) and since females (including those that are pupils) often apply high-scented perfumes, body creams and oils, they stand a higher chance of been repeatedly infected during water-contact activities. Though the work of Bigwan *et al.* (2013) had linked a higher prevalence of schistosomiasis in males to some socio-cultural practices such as bathing, washing, swimming, irrigation-farming and fishing in rivers or ponds, the reverse was the case in this study. Repeated infection episodes enhance the development of heavy infection or high intensity.

Individuals with heavy infections are most prone to chronic complications (Leder and Weller, 2011). By extension, the females are not only at a higher risk of developing complications like female genital schistosomiasis (FGS), but they stand the risks of decreased fertility, abortions, vaginal discharge, contact bleeding and increased risk of HIV infection due to underlying urinary schistosomiasis (Kjetland *et al.*, 2012).

The absence of urinary schistosomiasis in pupils within age-group of 4-6 years unlike in the older pupils could be due to the fact they are too young to engage in water-based activities (like swimming, wadding or 'Fadama' farming). Older pupils generally can engage freely in water activities (Bigwan *et al.*, 2013). Prevalence and intensity of urinary schistosomiasis significantly increased with increase in age of the pupils.Since exposure to risks of using unsafe water bodies is likely to occur in older pupils, re-infections are bound to happen and consequently increase the worm burdens in them and which is indirectly indicated by high means of egg count/10ml of urine. Other studies by Omenesa *et al.* (2015), Geleta *et al.*, 2015 have shown that pupils older age-groups are more infected with urinary schistosomiasis and that the infection increase with age of children.

The highest occurrence of urinary schistosomiasis among pupil in Bitaro, Ankun and Kwoi could be due to more availability of rivers and streams and the dense water-contact activities among the pupils compared to other locations within the LGA. Pupils in Samabang and Nok had an absence of the infection because there were fewer rivers and streams and reduced water-contact activities among the pupils. High water-contact activities promote a higher risk of contracting the infection. However, the highest mean count *of S. haematobium* eggs was found in Central Area and not in the location of highestprevalence of the infection. Hence, areas of high prevalence of the infection did

not coincide with high intensity of infection because repeated exposure is required to enhance the development of a heavy *Schistosoma* infection.

The overall occurrence of 2.0% heavy infections, 10.3% light infections and egg count ranging between 0–204eggs/10 ml of urine among the pupils was lower than the range of 21-1138eggs/10 ml of urine and 62.7% heavy infections reported by Ossai *et al.* (2014). This further indicated that pupils in Jaba LGA had relatively low burden of urinary schistosomiasis. Nevertheless, the infected population continues to pose risk to uninfected pupils and members of the community, which might increase the cost of personal and public health maintenance.

The 8.1%prevalence of anaemia among the pupils could have resulted from multiple sources: dietary deficiency of iron, leukaemia, heavy loss of blood and/or parasitic diseases. It had been noted by El-Hazmi and Warsy (2001) that haematological indices of apparently healthy individuals can be affected by certain factors like age, gender, cultural background, nature of body build, social activities, nutrition, altitude and other environmental factors. One of such haematological parameters considered in this study was PCV and classified as ‘anaemic’, ‘high’ or ‘normal’ as outlined by Cheesbrough (2009). Urinary schistosomiasis by statistics significantly caused and/or enhanced the development of anaemia among these pupils. Those who become recurrently infected may develop anaemia, malnutrition and even learning difficulties (CDC, 2012b). The anaemia can be related to the cumulative loss of blood in terminal haematuria associated with the disease (Grimes *et al.*, 2014) as well as the continuous feeding on glucose and blood products by schistosomes (Leder and Weller, 2011); hence,making urinary schistosomiasis a chronic symptomatic disease (King *et al.*, 2005). However, the cause of the anaemia in those pupils without urinary schistosomiasis in study may be due to other diseases of parasitic or non-parasitic origins.

In this study, pupils who wash their clothes in rivers and streams had higher occurrence (and 1.4 times at risk) of the infection than those who wash at home. Schistosomiasis has been reckoned as of one of the world's most prevalent public health problems (Bigwan *et al.*, 2013) and Nigeria stands as one of Africa's most endemic countries (Chitsulo *et al.,* 2000, Omenesa *et al.,* 205). Despite several control/intervention programmes, the prevalence of urinary schistosomiasis seemed not to be decreasing. This is partly due to improper implementation of those control measures, unawareness and increased activitiesin unsafe water bymembers of rural communities.

The complete unawareness of schistosomiasis among pupils in Jaba LGA is indicative of negligence of the disease in Nigeria, a characteristic that defines it as a 'neglected tropical disease (NTD)' in the condition of poverty (WHO, 2016). Hence, unawarenessof schistosomiasis among the pupilsin this study remains a strong risk factor in the continuous spread of the infection. A considerable level of 74.5%awareness of schistosomiasis by pupils in rural communities of Kano State, Nigeria (Dawaki *et al.,* 2015) led to a lower prevalence of 8.3% compared to the 12.3% prevalence obtained in this study among pupils that had noawareness of the infection and its risks. The pupils in Jaba LGA were at continued risk of getting infected because of unawareness and continuous engagement in activities in cercarie-infested-water bodies. But it is anticipated that the organized awareness talks on schistosomiasis in the pre-selected schools during this study will help to reduce the risks of exposure ofthe enlightened pupils.

The pupils who did not participate in swimming activity were more infected than those who were swimmers,though it was not statistically significant. Some other studiesby Omenesa *et al.,* (2015) and Dawaki *et al.,* (2015) had positively associated schistosomiasis with water-contact activities (like swimming) in riversorstreams. The

population that did not engage in swimming but had the disease in this study might have acquired the infections through other water-based activities such as washing or wading in rivers and streams or during irrigation farming.Hence, there are various waysschistosomes can be transmittedtothe skin/body once there is contact withinfested water.

Pupils' parents' level of formal education did not show any statistical relationship with urinary schistosomiasis in the pupils. However, the lowest prevalence was observed among pupils whose parents did not have any formal education. Perhaps, the tight working schedules of educated parents and late return from work gave their children/wards opportunities to indulge in unsafe water-contact activities. Contrarily, pupils whose mothers attained tertiary education had lower infection. The prevalence of the infection increased steadily among the pupils as their fathers' level of formal education increased. This finding did not agree with the work of Houmsou *et al.* (2012) in which children whose parents did not attain any formal education as well as those with primary education were most infected with*Schistosoma haematobium.*

Somesign and symptoms could be useful in the diagnosis of urinary schistosomiasis. Red/brown-coloured urine (a sign of haematuria) and painful micturition witnessed by some of the subjects in this study statistically indicated positive associations with urinary schistosomiasis. The association of haematuria with urinary schistosomiasis had been emphasized by Morenikeji *et al.*, (2014), and Dawaki *et al.*, (2015), and described as a classic or main sign of the disease (Van der Werf and de Vlas, 2004; WHO, 2016).

The egg of *Enterobius vermicularis*that was identified in the course of this study was observed in urine sample of a female pupil; it was indicative of enterobiasis. Eggs of this parasite are occasionally found in urine of young girls because female

adult(*Enterobius vermicularis*) worms migrate at night to the perianal and external genitalia of infected young girls to deposit their eggs, which may be carried in urine during micturition (Cheesbrough, 2006).

Concomitant bacteruria among the study population revealed seven different bacterial uropathogens of which *Citrobacter* spp and *Klebsiella* spp were the most prevalent. Many studies have implicated *E. coli* as the most occurring bacterial uropathogens. Uneke *et al.* (2009) reported that *E. coli* (19.0%) and *S. aureus* (31.8%) were the most prevalent uropathogens among children of Ngbo-West and Ezza-North LGAs respectively in Ebonyi-Nigeria. The presence of these bacterial uropathogens among the pupils can usher in episodes of UTI or they main remain asymptomatic. Urinary tract infections (UTIs) result from the invasion and colonization of tissues of parts of the urinary tract, with inflammatory responses; though asymptomatic cases occur but complicated cases are often discomforting (Bishop and Shehu, 2016).

# CHAPTER SIX

## 6.0CONCLUSION AND RECOMMENDATION

### 6.1 Conclusion

Jaba LGA in Kaduna State, Nigeria is considerably an area of mild burden of urinary schistosomiasis with a prevalence of 12.3% and overall mean intensity of 2.69±0.651 eggs/10ml of urine of pupils.

There was absence of *Salmonella* spp from the blood and urine samples of pupils examined. Hence, there was no co-infection between urinary schistosomiasis and *Salmonella* spp in this study.

The prevalence of anaemia among pupils in Jaba LGA was 8.1%;statistically, it was positively associated with concomitant urinary schistosomiasis($P < 0.05$). The anaemia was significantly increased in pupils with heavy burden of *Schistosoma haematobium* infection than in those with light infection.

SevendifferentGram-negative bacteria were isolated fromthe urine samples of the pupils, namely,*Acinetobacter* spp (6.7%), *Escherichia coli* (1.6%), *Citrobacter* spp (6.7%), *Klebsiella* spp(3.4%), *Providencia* spp (0.2%), *Pseudomonas aeruginosa* (0.4%) and *Serratia marcescens* (0.2%).However, *Citrobacter* spp (8.1%), *E. coli* (1.6%) and *Klebsiella* spp (1.6%) were found in co-infectionwith*Schistosoma haematobium*in this study. An egg of *Enterobius vermicularis*(0.2%) was detected in urine sample of a female pupil, indicating the presence of enterobiasis.

The female pupilshad higher prevalence and mean intensity of urinary schistosomiasis (15.5%, 4.18±1.202 eggs/10ml of urine)than the male pupilswho had a prevalence of 9.1% and mean intensity of 1.22±0.500 eggs/10ml of urine($P < 0.05$). Hence, the

female pupils are faced with heightened risks of developing some complications like female genital schistosomiasis (FGS) and irreversible infertility.

Prevalence and intensity of urinary schistosomiasis significantly increased with rise in age of pupils($P < 0.05$). Three villages, Bitaro (23.2%, 4.85±2.018eggs/10ml of urine),Ankun (22.2%, 3.30±2.301eggs/10ml of urine) and Kwoi (20.3%, 2.73±1.451eggs/10ml of urine) accounted for most of urinary schistosomiasis cases in Jaba LGA($P < 0.05$).

One of the factors that promoted the continuous spread of urinary schistosomiasis among pupils in Jaba LGA was lack of awareness of the disease; hence, more children stand at risk of the infection in the area. 'Fadama' farming was statistically associatedwith urinary schistosomiasis among the study population($P < 0.05$).This study raised a considerable level of awareness of schistosomiasis in all pre-selected schools in Jaba LGA, Kaduna State, Nigeria.

### 6.2 Recommendation

Schistosomiasis awareness campaigns in schools and communities should be initiated at all LGAs of endemic regions because children become infected majorly due to unawareness and use of unsafe water bodies. More researches on the possible effects of high intensity of urinary schistosomiasis on development of complications, especially in femalesubjects should be advocated.

In view of the impact of urinary schistosomiasis on health and development of African children, concerted participation of government, school authorities, parents and researchers should be aimedatprovision of safe water, control/prevention, treatment of risk groups, early reporting of research or case findings and vaccine development.

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**Appendix I: Questionnaire**

Sample No._____
Structured Questionnaire

**PREVALENCE OF URINARY SCHISTOSOMIASIS AND ITS CO-INFECTION WITH SALMONELLA SPECIES AMONG PUPILS IN JABA LGA, KADUNA STATE**

An MSc. Research in Microbiology
Department of Microbiology Faculty of Science, Ahmadu Bello University, Zaria, Nigeria

(A) SOCIO-DEMOGRAPHIC DATA

Risk factors

1. Sex: **Male** { } **Female** { }
2. Age: ……yrs
3. Primary: **1**{ }**2**{ }**3**{ }**4**{ }**5**{ }**6**{ }
4. Farming in "Fadama" lands: **Yes** { } **No** { }
5. Are you aware of schistosomiasis? **Yes** { } **No** { }
6. Swimming in river, stream, pond, dam **Yes** { } **No** { }
7. Do you fish in dam/river/stream? **Yes** { } **No** { }
8. Where do you wash your clothes? **At home{ } At River/ Stream** { } **At Dam** {}
9. Source of drinking water at home. **Well** { }**River/Stream**{ } **Dam**{ } **Borehole**{ } **Tap water**{ } B**ottled/water in sachet** { }
10. Father's highest level education: **None** { } **Primary** { } **Secondary** { }**Tertiary**{}
11. Mother's highest level of education: **None**{ } **Primary**{}**Secondary**{}**Tertiary**{}

Signs and symptoms

1. Presence of haematuria**Yes** { } **No**{ }
2. Pains during urination: **Yes** { } **No**{ }
3. Frequent urination: **Yes** { } **No**{ }
4. Do you feel abdominal pain? **Yes** { } **No**{ }
5. Do you have a fever? **Yes** { } **No**{ }

(B) LABORATORY ISOLATIONS AND IDENTIFICATIONS

**For laboratory use only** (Tick + if present or — if absent)

Urine colour: Cloudy { } Red and Cloudy { } Brown and Cloudy { } Yellow-Brown OrGreen-Brown { } Yellow Orange { } Milky-White { }

Presence of *Schistosoma haematobium* { } Egg count……../10ml of urine

Presence of *Salmonella* species…….serotype……Co-infection { }

Other parasites in urine…….PCV…….%

**Appendix II: Cultural Characteristics of Bacteria Isolated from Urine Samples of Pupils in Jaba LGA, Kaduna State, Nigeria**

| **Major Cultural Characteristics** | | **Isolate identity from** |
|---|---|---|
| **SSA** | **XLD** | **Appendix II** |
| Indistinct faintly pinkish colonies | Pale purplish colonies | *Acinetobacter*spp |
| Colourless to dark colonies | Light yellowish colonies, some with black centers | *Citrobacter*spp |
| Pinkish colonies | Large yellowish, yellowish red flat colonies | *Escherichia coli* |
| Large red/pinkish colonies | Viscous, mucoid, light-yellowish colonies | *Klebsiella* spp |
| No observed growth | Mucoid, reddish colonies | *Providencia*spp |
| Colourless pinhead colonies | Rough Pinkish colonies | *Pseudomonas aeruginosa* |
| Colourless mucoid colonies | Medium sized pinkish yellow colonies | *Serratia marcescens* |

**Appendix III: Biochemical Identification of Bacterial Isolates from Urine of Pupils from Jaba LGA, Kaduna State, Nigeria**

| Sample No. | Gram reaction (Gram negative) | TSI | $H_2S$ test | Indole | MR | VP | Citrate | Urease | Oxidase | Motility | LDC | ODC | Identity of Isolate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NB5 | Coccobacilli | K/NC | - | - | - | - | + | + | - | - | - | - | *Acinetobacter*spp |
| HB4 | Coccobacilli | K/NC | - | - | - | - | + | + | - | - | - | - | *Acinetobacter*spp |
| ND4 | Coccobacilli | NC/NC | - | - | - | - | + | + | - | - | - | - | *Acinetobacter*spp |
| HD5 | Coccobacilli | K/NC | - | - | - | - | + | + | - | - | - | - | *Acinetobacter*spp |
| HF4 | Coccobacilli | K/NC | - | - | - | - | + | + | - | - | - | - | *Acinetobacter*spp |
| BB6 | Coccobacilli | K/NC | - | - | - | - | + | + | - | - | - | - | *Acinetobacter*spp |
| YE5 | Coccobacilli | K/K | - | - | - | - | - | $+^{w}$ | - | - | - | - | *Acinetobacter*spp |
| YE8 | Coccobacilli | K/K | - | - | - | - | + | + | - | - | - | - | *Acinetobacter*spp |
| HE2 | Coccobacilli | K/NC | - | - | - | - | + | + | - | - | - | - | *Acinetobacter*spp |
| HF1 | Coccobacilli | K/NC | - | - | - | - | + | + | - | - | - | - | *Acinetobacter*spp |
| CD2 | Paired rods | $A/A_{+G}$ | - | + | + | - | + | + | - | + | - | - | *Citrobacter koseri* |
| CE7 | Rods | $A/A_{+G}$ | - | + | + | - | + | + | - | + | - | + | *Citrobacter koseri* |
| CF2 | Paired rods | $A/A_{+G}$ | - | + | + | - | + | $+^{w}$ | - | + | - | + | *Citrobacter koseri* |
| YF4 | Paired rods | $A/A_{+G}$ | - | + | + | - | + | + | - | + | - | - | *Citrobacter koseri* |
| AB1 | Paired rods | $A/A_{+G}$ | - | + | + | - | + | + | - | + | - | + | *Citrobacter koseri* |
| AD3 | Paired rods | $A/A_{+G}$ | - | + | + | - | + | + | - | + | - | - | *Citrobacter koseri* |
| CB2 | Paired rods | $A/A_{+G}$ | - | + | + | - | + | + | - | + | - | + | *Citrobacter koseri* |
| AC5 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| CA10 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | - | *Citrobacter* spp |
| CD6 | Rods | K/A | - | - | + | - | + | - | - | + | - | - | *Citrobacter* spp |
| CE2 | Rods | A/A | - | - | + | - | + | - | - | + | - | - | *Citrobacter* spp |
| CE5 | Paired rods | $A/A_{+G}$ | + | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| CE6 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| CE8 | Paired rods | $A/A_{+G}$ | + | - | + | - | + | + | - | + | - | - | *Citrobacter* spp |
| HA5 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| HC1 | Paired rods | A/A | - | - | + | - | - | + | - | + | - | + | *Citrobacter* spp |
| HE3 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| NA1 | Rods | A/A | - | - | + | - | + | + | - | + | - | - | *Citrobacter* spp |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NA2 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| NB1 | Paired rods | A/A | - | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| NB7 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| NC1 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| NC6 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | - | *Citrobacter* spp |
| NF4 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| SA11 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| SA3 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| SA5 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | - | *Citrobacter* spp |
| SC16 | Rods | K/A | - | - | + | - | + | + | - | + | - | - | *Citrobacter* spp |
| KE8 | Paired rods | $A/A_{+G}$ | + | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| SE8 | Rods | $A/A_{+G}$ | + | - | + | - | + | + | - | + | - | - | *Citrobacter* spp |
| SF1 | Rods | A/A | - | - | + | - | + | + | - | + | - | + | *Citrobacter* spp |
| SF4 | Rods | $A/A_{+G}$ | - | - | + | - | + | + | - | + | - | - | *Citrobacter* spp |
| YD10 | Rods | $A/A_{+G}$ | - | - | + | - | + | $+^{w}$ | - | + | - | + | *Citrobacter* spp |
| CD7 | Rods | $A/A_{+G}$ | + | + | - | + | + | - | - | + | - | + | *Citrobacter* spp |
| CE3 | Rods | $A/A_{+G}$ | - | + | + | - | - | - | - | + | + | + | *E. coli* |
| NB3 | Rods | $A/A_{+G}$ | - | + | + | - | - | - | - | + | + | + | *E. coli* |
| ND1 | Rods | $A/A_{+G}$ | - | + | + | - | - | - | - | + | + | + | *E. coli* |
| SD12 | Rods | $A/A_{+G}$ | - | + | + | - | - | - | - | + | + | + | *E. coli* |
| YC7 | Rods | $A/A_{+G}$ | - | + | + | - | - | - | - | + | + | + | *E. coli* |
| YD1 | Rods | $A/A_{+G}$ | - | + | + | - | - | - | - | + | + | - | *E. coli* |
| YD11 | Rods | $A/A_{+G}$ | - | + | + | - | - | - | - | + | + | + | *E. coli* |
| YE4 | Rods | $A/A_{+G}$ | - | + | + | - | - | - | - | + | + | - | *E. coli* |
| BA3 | Small rods | $A/A_{+G}$ | - | + | - | + | + | + | - | - | + | - | *Klebsiella oxytoca* |
| SE3 | Small rods | $A/A_{+G}$ | - | + | - | + | + | + | - | - | + | - | *Klebsiella oxytoca* |
| CD1 | Small rods | $A/A_{+G}$ | - | + | - | + | + | + | - | - | + | - | *Klebsiella oxytoca* |
| CD3 | Short rods | $A/A_{+G}$ | - | + | - | + | + | + | - | - | + | - | *Klebsiella oxytoca* |
| CC8 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| CD5 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| NA5 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| NE4 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| SB1 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| SB6 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| SB7 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| SD15 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| KC5 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| YD4 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| YE12 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| YE3 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| NF2 | Short rods | $A/A_{+G}$ | - | - | - | + | + | + | - | - | + | - | *K. pneumoniae* |
| NB6 | Rods | $A/A_{+G}$ | - | + | + | - | + | + | - | + | - | - | *Providencia* spp |
| ND5 | Rods | K/K | - | - | - | - | + | - | + | + | - | - | *P. aeruginosa* |
| NE3 | Rods | K/K | - | - | - | - | + | - | + | + | - | - | *P. aeruginosa* |
| HC2 | Rods | K/A | - | - | - | + | + | + | - | + | - | - | *Serratia marcescens* |

Keys: + (means positive reaction); - (means negative reaction); w (weak);A: Yellow or Acidic; G: gas, $H_2S$: Hydrogen sulphide, K: Red or alkaline, MR: Methy red,

NC: No change, LDC: Lysine decarboxylation, ODC: Ornithine decarboxylation, TSI: Sugar fermentation in Triple Sugar Iron agar, VP: Voges Proskauer (Constantiniu*et al.*, 2004; Global Health Laboratories, 2005; Cheesbrough, 2006; Willey *et al.*, 2008).

**Appendix IV: Various Stages of Activities involved in the Study at Jaba LGA, Kaduna State, Nigeria**

(a) Creation of awareness on urinary schistosomiasis at Gora, Jaba LGA.
(b) Participated volunteers and their teachers at Chori, Jaba LGA.
(c) Instruction on how to collect urine samples at Central Area, Jaba LGA.
(d) Issuance of laboratory tests results to pupils witnessed by their teacher at Yadi-Pyok, Jaba LGA.

**Appendix V: Ethical Approval by Kaduna State Universal Basic Education Board**

KADUNA STATE UNIVERSAL BASIC EDUCATION BOARD (SUBEB)

HEAD OFFICE:
79A TAFAWA BALEWA WAY,
P.M.B. 2333 KADUNA,
KADUNA STATE.
E-mail:kadunasubeb@yahoo.com

Ref: KD/SUBEB/380/VOL.I/8

Date: 9TH April, 2015

The Education Secretary,
LGEA,
Jaba.

RE: LETTER OF INTRODUCTION: HENRY GABRIEL BISHOP (P13SCMC 8027)

I am directed to refer to the attached letter on the above subject, dated 24th March, 2015 from ABU Zaria and to request you to give him maximum cooperation, please.

Theresa M. Wakili
For: Executive Chairman

**Appendix VI: Ethical Approval by Kaduna State Ministry of Health**

# MINISTRY OF HEALTH, KADUNA STATE

All Communication to be addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Telephone: 234-248048
Website: ttp://www/moh.kd.gov.ng.
Email: info@moh.kd.gov.ng.

Independence Way,
P.M/;B 2014
Kaduna.
Kaduna State, Nigeria.

MOH/ADM/744/VOL.I/290

4th June, 2015

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**NOTICE OF APPROVAL AFTER FULL COMMITTEE REVIEW**

**RE: CO - INFECTION OF SCHISTOSOMA HAEMATOBIUM AND SALMONELLA SPECIES IN PUPILS AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF SALMONELLA SPECIES**

| | | |
|---|---|---|
| Name of Principal Investigator | - | **Henry Gabriel Bishop** |
| Address of Principal Investigator | - | **Faculty of Science, Dept. of Microbiology, Ahmadu Bello University, Zaria Kaduna State.** |
| Date of Receipt of Application | - | **25th March, 2015** |
| Date of Ethical Approval | - | **30th April, 2015** |

This is to inform you that the research described in the submitted protocol, the consent forms, advertisements and other participant information materials have been reviewed and given full approval by the Health Research Ethics Committee.

If there is delay in starting the research, inform the HREC so that the dates of approval can be adjusted accordingly.

However, researcher is kindly requested to submit a copy of his/her findings to the state Ministry of Health, please.

**DR. B. M. JATAU**
Chairman
Research Ethics Committee