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# Evaluation of the Efficacy of Microscopy and PCR in the Diagnosis of Urinary Schistosomiasis among Primary School Children in Wamakko Local Government Area of Sokoto State – Nigeria

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors THIS, EJE and KM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MFU and NDN managed the analyses of the study. Author FPNU managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The aim of this study was to evaluate the efficacy of microscopy and PCR in the diagnosis of urinary schistosomiasis among primary school children in Wamakko Local Government Area. Sokoto, North-western, Nigeria.

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**Study Design:** This was a cross-sectional, descriptive study designed to evaluate the efficacy of microscopy and Polymerase Chain Reaction in the diagnosis of urinary schistosomiasis among primary school children in Wamakko Local Government Area. Sokoto, North-western, Nigeria.
**Place and Duration of Study:** This research was conducted at the centre for advance research and training Usmanu Danfodiyo University, Sokoto, Sokoto State, from May to July, 2016.
**Methodology:** Study was carried out on randomly selected 50 urine samples of Primary school children in Wamakko. The DNA extraction was carried out using the phenol-chloroform method and the extracted DNA was amplified by PCR using a set of primers to detect *Dra* 1 repeat gene fragment at 121bp. The PCR products were detected using Agarose gel electrophoresis.
**Results:** Among the study population, *S. haematobium* eggs were detected in 5 subjects while *S. haematobium* DNA was detected in 3 subjects. One of the subject was positive with PCR but negative with microscopy. The sensitivity and specificity for PCR were 60.0% and 95.92%, respectively.
**Conclusion:** From the result presented, even-though there are only five samples (5) positive for *Schistosoma haematobium* but conclusion cannot be made that microscopy is the gold standard but that, it is suitable for diagnosis of acute urinary schistosomiasis in children Due to high cost, PCR should be reserved for clinically susceptible cases, areas of low endemicity and light infection.

*Keywords: Efficacy of microscopy and PCR; urinary schistosomiasis; children; Sokoto State, Nigeria.*

## 1. INTRODUCTION

Schistosomiasis, originally known as Bilharziasis is a parasitic disease caused by the platy helminth worm of the class Trematoda, genus *Schistosoma* (commonly known as blood-flukes) and is relatively common in developing countries, especially on the continent of Africa and in central rural zones of Egypt and China [1]. In 1851, Theodor Bilharz discovered parasites in portal circulation and associated them with being causal agents of cystitis. Pirajá da Silva first described the pathogenic agent in 1908. Ferguson, in 1911, was the first to report the high frequency of bladder cancer and its etiological relation to urinary bilharziasis [2].

Schistosomiasis is the second leading parasitic challenge to public health after malaria. There are 5 species of *Schistosoma* parasites that produce schistosomiasis in humans and each of them has its respective clinical manifestations [3]: *Schistosoma mansoni* and *Schistosoma intercalatum* cause intestinal schistosomiasis, *Schistosoma japonicum* and *Schistosoma mekongi* cause the Asian variety of intestinal schistosomiasis, and *Schistosoma haematobium* causes urinary schistosomiasis and is most frequent in Africa, the Middle East and the Mediterranean [1]. Schistosomes infect approximately 200 million people in the tropics and subtropics, and of these, *S. haematobium* infects close to 100 million people in 53 countries of Africa and the Middle East. Schistosomiasis is widely distributed in Nigeria. It is hyper-endemic in many states of the north and southwest with moderate to low endemicity in the southeast. *S. haematobium* occurs in nearly all endemic foci while *S. mansoni* is predominantly found in the north and some part of the southwest [4]. Like other neglected tropical diseases, urinary schistosomiasis is endemic in poor and marginalized Communities [5].

Diagnosis is made through the study of urinary sediment in the search for eggs. Urine were collected between 11:00 and 14:00 hours, considered to be the hours in which there is maximum parasite expulsion. Infection is classified as mild when there are less than or equal to 50 eggs per 10 mL and serious when there are more than 50 eggs per 10 mL [6].

Despite the numerous available diagnostic technique employed in the diagnosis of urinary schistosomiasis, some practical issues are yet to be clarified regarding the basic diagnostic tools associated with this public health important parasitic disease. The direct microscopic sedimentation technique of urine and examination of urine using filtration method for the detection of *S. haematobium* egg, including indirect methods i.e. detection of haematuria, proteinuria, circulating egg antigens, schistosome specific antibodies and ultrasound scans etc are among the diagnostic techniques [7]. Membrane use in urine filtration remains a common egg concentration technique which thereafter, enables counting of eggs under the microscope. Hence, microscopy is the gold standard in the diagnosis of urinary schistosomiasis [8].

Several Studies had been conducted in detecting *S. heamatobium* using polymerase chain reaction. Hamburger and his colleague cloned *S. haematobium* genome repeated sequence, using the Dra I repeated sequence [9].

The polymerase chain reaction is a powerful technique that has rapidly become one of the most widely used techniques in molecular biology because it is quick, sensitive and specific. The technique utilises DNA polymerase enzymes and synthetic oligonucleotides to produce billion copies of specific regions of DNA. Polymerase Chain Reaction have proven to be highly sensitive and specific molecular tool for detecting *S. haematobium* [10].

Investigators have launched molecular approaches for detecting schistosomiasis; detection of parasite (schistosome) DNA in clinical human samples such as faeces, sera and urine, confirms the existence of parasite DNA in the host and provide evidence of the infection. Parasite DNA may be a helpful guideline for selecting the appropriate treatment for schistosomiasis [11]. A considerable amount of literature has been published on PCR methods and is proven to have improve the direct detection of *S. haematobium*. DNA seems to be prepared from egg prior to PCR amplification from urine, stool or organ biopsy as samples. Mitochondrial genes have been used in molecular analysis to demonstrate the relationship between different species, because they show high mutation rates compared to nuclear markers and exist in high number. More-over, this led to their exploitation for studying phylogenetic variation in these parasites [12].

## 2. METHODOLOGY

### 2.1 Study Design

Cross sectional study was conducted in Wamakko Local Government Area, Sokoto State, from May to July, 2016. The study was performed by collecting urine samples as well as the administration of questionnaire to the participants.

### 2.2 Study Area

Wamakko is located in Sokoto State of Nigeria. It is one of the 23 Local Government Area that make up the present Sokoto State**.** The selected area for this study is located at the extreme North West of Nigeria between longitude 05° $11^1$ to 13° $03^1$ East and Latitude 13° 00 to 13° $06^1$ North. It covers an area of 60.33 km square, with an annual rainfall ranging between 500 mm to 1300 mm [13]. Dry season starts from October and last up to May and wet season begins in May and last up to September every year [13]. The predominant tribes of Wamakko are: Fulani, Hausa and some of the minority tribes such as Yoruba, Igbo etc. the inhabitants major occupation include: rearing of animals, fishing and petty trading [14].

### 2.3 Study Population

The study population includes both male and female children attending primary school in Wamakko whose age range between 5 to 16 years old.

### 2.4 Inclusion Criteria

Participation in the study was based on pupils' willingness and interest. Also, random sampling method was employed to include primary school children between 5 – 16 years of age.

### 2.5 Exclusion Criteria

Primary school children that were unwilling and uninterested to participate, as well as pupils outside the age range of 5 – 16 years old.

### 2.6 Ethical Consideration

Objectives and procedures of the research were explained to the various school heads and the participants. An informed consent was obtained from all the respondents and the various school heads before inclusion using approved protocol. Approval was also obtained from the ethical committee of Sokoto State Ministry of Health.

### 2.7 Sample Selection

Urine samples were collected into well labelled, sterile, leaked proof screw caped universal container between 10:00 to 14:00 hours for optimal egg passage.

### 2.8 Filtration Technique

Approximately, 10 ml of urine sample passed through Whatman No 1 (What-man international, Maidstone England) filter paper. The filtration was accomplished by vacuum pump filtration.

(Bedford cooperation, Masachussel, USA. The exposed quadrant of the filter paper which contain the eggs were stained with 1% ninhydrine in acetone solution as well as lugols iodine and allowed to develop overnight. This helped to increase the visibility of the eggs. Thereafter, the filter paper was examined under the microscope using x10 objective lens to check for the presence or absence of the ova of *S. haematobium* [15].

## 2.9 Extraction of DNA

The DNA extraction was carried out using the phenol-chloroform method [16]. One millimetre (1 mL) of urine sample was spun in an Eppendorf tube at 12,000 rpm using high speed refrigerated micro-centrifuge (Model MX-301 Tokyo, Japan). The supernatant was discarded and the deposit re-suspended. 250 µL of lysis buffer was placed in another Eppendorf tube and 250 µL of the re-suspended sediment was added. The lysate was centrifuged at 12,000 rpm for 5 minutes. 250 µL of the lysate was added to another Eppendorf tube containing 250 µL of buffered phenol/ chloroform/iso-amyl alcohol and 25µL of 3M sodium acetate. It was vortexed (XH-B) for 1 minute. It was incubated on ice for 15 minutes. It was centrifuged at 12,000 rpm for 10 minutes. 250 µL of the aqueous layer was picked and added to a tube containing 250 µL of isopropanol. It was incubated at -20°C for 5 minutes to precipitate DNA. The supernatant was discarded and the pellets were suspended in 500 µL of 70% ethanol. It was vortexed and centrifuged at 12,000 rpm. The supernatant was discarded and the deposit was spun quickly to remove residual ethanol. The deposit was air dried in a half opened tube for 30 minutes. 20 µL of Tris EDTA was added to the tube and re-suspended to elute the DNA. The purity and concentration of extracted DNA was checked using Spectrophotometer (Bioscience Nano, Shimadzu Corporation).

## 2.10 Materials for PCR Amplification of Schistosome *Dra* 1 Repeat Sequences

The following materials were used: Nuclease free water, 5X master-mix. *Dra 1* repeat: Forward primers $5^1$GATCTCACCTATCAGACGAAAC$3^1$ and reverse primers 5'TCACAACGATACGACCAAC $3^1$ (Jena Bioscience, Germany).

A modified technique of [17] Hassan et al., was used for the PCR in this study. The genomic DNA was subjected to PCR amplification of the schistosome *Dra1* repeat sequence, using forward primers $5^1$ GATCTCACCTATCAGACGAAAC$3^1$, and reverse primers, 5'TCACAACGATACGACCAAC $3^1$.

These were used to detect the presence of *S. haematobium*, yielding a PCR product of 121bp. For specific amplification of *S. haematobium*, the mix for the PCR was performed in a 50 µL reaction mixture containing 10 µl of 5X master mix (Jena Bioscience, Germany), 1.5 µL of forward primers, 1.5 µL reverse primers, 1.5 µL of dimethylsulphoxide, 15.5 µL nuclease free water and 20 µL of the DNA template.

The PCR cycling conditions comprised of initial denaturation at 95°C for 5 minutes, followed by 33 cycles of 95°C for 30 seconds (denaturation), annealing at 53°C for 90 seconds and extension at 72°C for 1 minute and final extension at 72°C for 5 minutes. It was thereafter, kept on hold at 4°C. The DNA amplification was carried out using Kyratec Super thermal cycler (Model SC 300, Australia).

## 2.11 Agarose Gel Electrophoresis

The PCR products were electrophoresed in 2% agarose gel (the gel containing 5 µl of 10 mg/ml of ethidium bromide solution) at 120V for 90 minutes. 100 bp (DNA marker (Promega Madison Wi, USA) was used as the molecular size marker. The documentation was done using Scope 21 UV transiluminator Desktop Gel Imager (Model UVT1513, Tokvo-Japan).

## 3. RESULTS

Cross sectional experimental study was conducted to evaluate the efficacy of Polymerase Chain Reaction in the diagnosis of urinary schistosomiasis among primary school children in Wamakko Local Government Area of Sokoto state, Nigeria.

Table 1 shows the sensitivity and specificity of PCR in comparison with microscopy, using 50 urine samples of primary school children. The table showed that; Sensitivity was 60.0%, Specificity (95.92%), Positive predictive value (60.0%) and Negative predictive value (51.09%).

**Table 1. Sensitivity and specificity of PCR in comparison with microscopy, using fifty (50) urine samples of primary school children**

| Variable | Number positive | Number negative | Total |
|---|---|---|---|
| Microscopy | 5 | 45 | 50 |
| PCR | 3 | 47 | 50 |

*Sensitivity = 60.0%*
*Specificity = 95.92%*

Fig. 1 shows M-100 bp Molecular marker (Promega), PC-Positive control, NC –Negative control, 1-10 PCR products of DNA samples from urine of primary school children targeting DRA-1 repeat gene of *Schistosoma Hematobium*; samples 7 and 10 are positive showing the amplified 121bp segment homologous to the target sequences.

Fig. 2 shows M-100bp Molecular marker (Promega), PC –Positive control, NC- Negative control, sample 11 was negative with microscopy but positive with PCR, showing the amplified 121 bp segment homologous to the target sequences of DRA-1 repeat gene of *Schistosoma hematobium*.

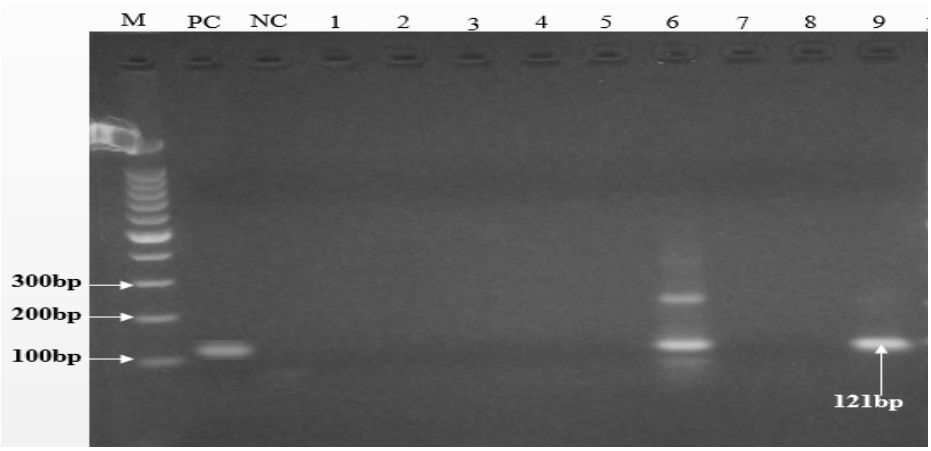


**Fig. 1. M-100 bp Molecular marker (Promega), PC –Positive control, NC- Negative control, 1-9 PCR products of DNA samples from urine of primary school children targeting DRA-1 repeat gene of *Schistosoma hematobium*; samples 7 and 10 are positive showing the amplified 121 bp segment homologous to the target sequences**

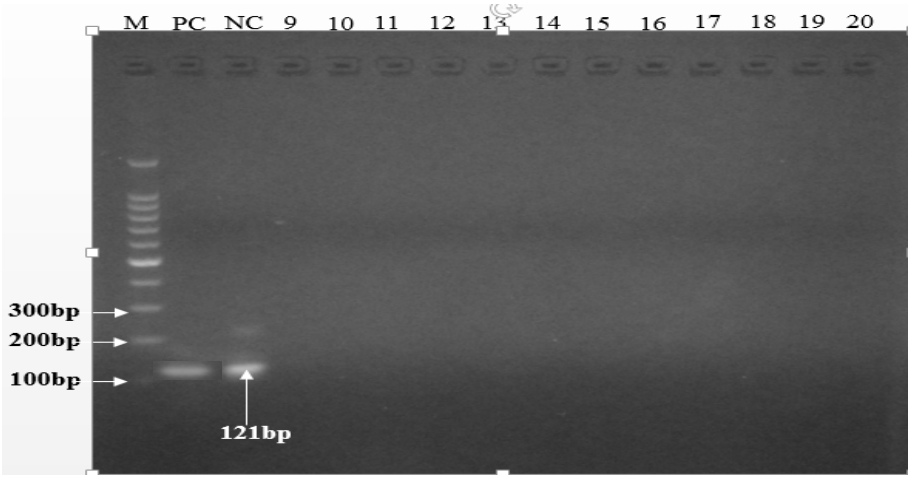


**Fig. 2. M-100 bp Molecular marker (Promega), PC –Positive control, NC-Negative control, sample 11 was negative with microscopy but positive with PCR, showing the amplified 121 bp segment homologous to the target sequences of DRA-1 repeat gene of *Schistosoma hematobium***

## 4. DISCUSSION

Diagnostic performance of microscopy and PCR: it is obvious that any environmental setting aimed at eradicating schistosomiasis should consider issues related to detection. This is because most of the commonly used methods may lack necessary sensitivity and specificity to accurately come with prevalence of schistosomiasis or parasitic burden [18].

Fifty urine samples were randomly selected for the PCR study. Samples 6 – 10 (Fig. 2) were positive for urinary Schistosomiasis. While others were negative. M-100 bp Molecular marker (Promega USA), PC-Positive control, NC – Negative control, 1-10 PCR products of DNA samples from urine of primary school children targeting DRA-1 repeat gene of *S. hematobium*; samples 7 and 10 were positive showing the amplified 121 bp segment homologous to the target sequences. M-100 bp Molecular marker (Promega), NC –Negative control, sample 11 was negative with microscopy but positive with PCR, showing the amplified 121 bp segment homologous to the target sequences of DRA-1 repeat gene of *S. hematobium*.

Findings of the study shows that, the Sensitivity of PCR was 60.0% while the specificity was 95.9%. This does not mean that PCR is not sensitive. Sensitivity, specificity, as well as positive predictive value and negative predictive value are influenced by the prevalence of disease in the population that is being tested. From this study, out of the 50 school children that were randomly selected, 5 were positive with microscopy while 3 were positive with PCR. This very low prevalence is responsible for the low sensitivity value of PCR in the study. A similar study was conducted by [19] who reported a sensitivity of 95.4% and specificity of 96%. And a sensitivity of 100% and a specificity of 60.5% were also reported [20].

From the results of the study, out of the 5 samples were positive with microscopy, 2 were positive with PCR. Furthermore, one of the samples was positive with PCR but negative with microscopy. It could be that the eggs of *S. haematobium* may not always be the only species of *Schistosomes* that might be found in the urine samples of primary school children in Wamakko Local Government Area, The following Schistosomes might be implicated; *S. bovis, S. intercalatum, etc.* microscopically the ova of *S. bovis, S. intercalatum* look alike under the microscope. Also, in Wamakko Local Government Area where this research was conducted, majority of the Farmers were herdsmen and their cattles drink from the same river where the primary school child have contact with. This is in corroboration with the report of [21] *Schistosoma bovis* is a blood fluke in cattle. It is one of the *Schistosoma* species that share a similar life cycle with S. *haematobium* group of Schistosomes. Hybrids between *S. bovis* and *S. haematobium* were first described in 2009 in Northern Senegalese so they are epidemiologically important. Other species of *Schistosoma* include: *S. mansoni, S. japonicum, S. intercalatum, S. mekongi, S. malayensis* etc. [22]. The *Dra* 1 repeat primers that were used for this study, are very sensitive and specific to only *S. haematobium.* Sensitivity is the probability that a test result will be positive when the disease is present, specificity is the probability that a test result will be negative when the disease is not present. Positive predictive value is the probability that the disease is present when the test is positive while negative predictive value is the probability that the disease is not present when the test is negative.

## 5. CONCLUSION

From the result presented, even-though there are only five samples (5) positive for *Schistosoma haematobium* but conclusion cannot be made that microscopy is the gold standard but that, it is suitable for diagnosis of acute urinary schistosomiasis in children. Due to high cost, PCR should be reserved for clinically susceptible cases, areas of low endemicity and light infection, adult samples, where there is doubt about the result of microscopy, presence of other species of *Schistosoma* (*S. bovis, S. intercalatum*) other than *S. haematobium*, as well as where there is cross breeding between *S. haematobium* and other species.

Polymerase Chain Reaction (PCR) remains most reliable and highly efficacious laboratory investigative tool for the diagnosis of urinary schistosomiasis caused by *S. haematobium.* The eggs of *S. haematobium* may not always the only species of *schistosomes* that might be found in the urine samples of primary school children in Wamakko Local Government Area, The following schistosomes might be implicated; *S. bovis, S. intercalatum,* etc.

## 6. RECOMMENDATIONS

Where possible, PCR should be used to confirm the result obtained from microscopy in order to be able to ascertain the species of schistosome that is responsible for such urinary Schistosomiasis. This is because the eggs of *S. haematobium, S. bovis, S. intercalatum* etc. may be implicated. Furthermore, encouragement of molecular research and studies that will employ the use of different primers for the diagnosis of the various schistosomes that could be found in urine. Sequencing of the urinary schistosomes found in Wamakko Local Government Area.

## CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

## ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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