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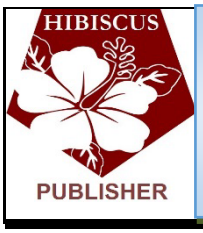

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Epidemiology and Molecular Study of *Schistosoma haematobium* Infection Among People in Riverine Communities of Sokoto State, Nigeria

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ABSTRACT

Microscopic examination of urine samples from individuals has long been regarded as the gold standard for diagnosing *Schistosoma haematobium* infection in Nigeria. However, the reliability of this method has been questioned. In light of this, the present study was designed to conduct an Epidemiology and Molecular Study of *S. haematobium* Infection Among People in Riverine Communities of Sokoto State. A total of 900 urine samples were collected. These samples underwent microscopic examination after filtration. Positive samples underwent DNA extraction, followed by PCR analysis to detect the presence of *Schistosoma haematobium* at 121 base pairs. Microscopically, 315 out of 900 samples (35.0%) tested positive for *Schistosoma haematobium*, with a mean egg intensity of 45.3 eggs per 10 mL of urine. The prevalence was notably high among fishermen (57.1%), individuals using any available water source (100.0%), visitors to freshwater bodies (37.7%), those residing very close to freshwater bodies (41.5%), and individuals who urinated indiscriminately (70.0%). The intensity of infection was significantly elevated among housewives (56.5%), visitors to freshwater bodies (58.7%), and individuals who urinated directly into freshwater bodies (57.2%). PCR analysis further confirmed the presence of *Schistosoma haematobium* in 87.6% of the samples previously analyzed microscopically. Infection rates were particularly significant among fishermen (98.2%), individuals using rivers as their water source (99.1%), visitors to freshwater bodies (89.8%), swimmers (97.8%), those residing very close to freshwater bodies (98.6%), and individuals who urinated directly into freshwater bodies (98.9%). The study revealed that microscopic examination alone may not be entirely effective in the study area. Therefore, the adoption of binary diagnosis methods is strongly recommended for more efficient detection of *Schistosoma haematobium* in the State.

INTRODUCTION

Schistosoma haematobium is a parasitic fluke belonging to the Super Kingdom; Eukaryota, Kingdom; Animalia, Phylum; Platyhelminthes, Class; Trematoda, Genus; *Schistosomes* [1]. The parasite is responsible for the cause of the most dangerous neglected tropical diseases (NTDs) in Africa [2]. These days, some estimates compare the global health impact of schistosomiasis to that of tuberculosis or malaria because of the higher rate of re-emergence and rise in cases [3]. A Schistosoma infection can cause significant illness, severe impacts on adult productivity and childhood development, and sometimes even death. This is because the parasites produce resistant antigens that can cause permanent damage to a variety of organs [4].

Schistosomes typically live between three and ten years, although they can sometimes live up to forty years in the bodies of humans [5]. The majority of adult male and female worms live in pairs or copulas. The female's slim body fits into the male's gynaecophoric canal, where she deposits her eggs, and the male

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fertilizes them [6]. The majority of the energy used by adult worms to digest erythrocytes comes from the breakdown of sugar. The oxidative degradation of fatty acids is necessary for the generation of eggs, and both glucose and fatty acids are obtained from the host [7]. The worms reside in the mesenteric or perivascular venous vessels of the infected individuals [8]. Because schistosomes lack an anus and are unable to expel waste, they regurgitate excrement into the infected person's circulation [9].

Most cases of snail fever occur in rural areas that sustain inland fisheries and agriculture in Asia, South America, and Africa [10]. Also, six species of schistosomes that are responsible for human schistosomiasis include; *Schistosoma haematobium* which causes urinary schistosomiasis, while *S. mansoni, S. japonicum, S. intercalatum, S. mekongi,* and *S. guineensis* cause intestinal schistosomiasis [11].

Africa and the Middle East are home to both *S. haematobium* and *S. mansoni*, while only *S. mansoni* is found in America [12]. Asia is home to *S. japonicum*, with China and the Philippines being its main locations [16]. The Mekong River watershed is home to three more locally distributed species, including *S. mekongi*, that also cause human schistosomiasis. On the other hand, West and Central Africa are home to *S. guineensis* and *S. intercalatum* [13].

According to reports, each species of *Schistosoma* has a particular range of acceptable snail hosts; as a result, the distribution of Schistosomes is determined by the ecological region of their host mollusk [18]. While *S. japonicum* utilises amphibious freshwater *Oncomelania* spp. as an intermediate host, *S. mansoni* and *S. haematobium* require specific species of aquatic freshwater *Biomphalaria* and *Bulinus* snails, respectively [14].
Approximately 779 million individuals globally are susceptible to Schistosoma infection in endemic regions, and over 250 million people are thought to be infected with *S. haematobium* at this time [21]. Nearly 90% of infections have been documented cases, the majority of which are localised in Africa [15]. Schistosomiasis results in around 3.3 million impairments and 11,700 fatalities yearly in impoverished nations including Senegal, Ghana, Nigeria, and Kenya [16].

Although schistosomiasis had been fully eradicated in most Nigerian states, including Sokoto, according to a 2012 report from the Federal Ministry of Health (FMH) [17], there had been a greater recurrence of the illness in Sokoto State [18,19,20].
S. haematobium was found to be the most pathogenic, however, in most cases, the diagnosis of the parasite infection was made by microscopic examination of the parasites' eggs in urine samples [21]. While evaluation for the specificity effect of the microscopy was scanty in the riverine community of Sokoto State, in spite, it was not 100% efficient in most endemic areas.
Therefore, to produce the most reliable results on the current status of Urinary schistosomiasis in Sokoto State, the Present study was designed to combine microscopy and molecular (PCR) methods for the best prevention and control of Urinary Schistosomiasis in the state.

MATERIALS AND METHODS

Study area

This study was conducted in riverine communities in Sokoto State, which is located in Nigeria's extreme northwest and has a land area of 28,232.37 square kilometres. Its coordinates are 13° 0' 21.1428" N and 5° 14' 51.1872" E. The state borders Zamfara State to the south, Kebbi State to the west and north, and the Republic of the Niger to the east. According to Topographic Sheet [22], Sokoto is the state's capital and largest city. According to NPC [23], the state's population is expected to be more than 4.2 million. The majority of the population in Sokoto state is Hausa/Fulani, although there are also significant populations of Yoruba, Igbo, Kabawa, Zamfarawa, and Zabarmawa; other tribes including Dakkarawa, Igala, Ibra, Nufawa, and Gwarawa are also residing in the state due to the area's several occupations [24].

Socio-environmental context of Sokoto State

According to Kasim and Usman [25], the average temperature of Sokoto State, which is in the arid Sahel, is 28.3°C (82.9 °F). Despite the area's typical high temperatures, the dryness keeps them below 40°C (104.0°F) for most of the year, the warmest months are February through April when daytime highs can reach 45°C (113.0°F). The rainy season, which runs from June to October, is dominated by the harmattan wind, which blows Sahara dust across the area and decreases temperatures by blocking sunlight and causing dust to build up in buildings [26].

The leading diseases in the area are malaria, typhoid, hypertension, schistosomiasis, hepatitis, cholera, syphilis, gonorrhea, tuberculosis, filariasis, trichomoniasis, loasis, HIV, ascariasis, and trichomoniasis. However, malaria, typhoid, hypertension, and schistosomiasis cause the highest morbidity and mortality in the State [27]. People in these areas are mostly Hausa/Fulani in tribe and Muslims in religion; farming, fishing, and herding are the predominant occupational activities in the areas; however, there are businessmen, civil servants, students, and housewives. Boreholes and wells are scanty for the people in these areas, people totally/partially depend on dams, rivers, or lakes for their occupational and chore activities including watering animals, irrigation farming, fishing, washing, swimming, cooking, drinking, etc.

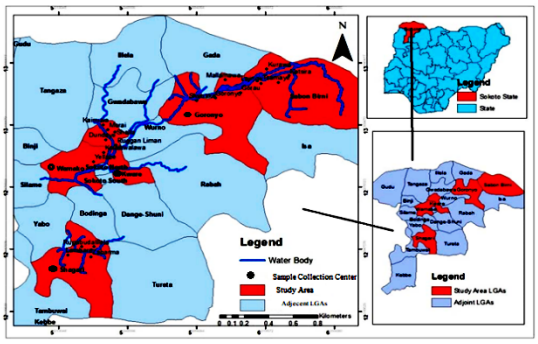

Fig 1. Map of the study areas (2022 Geographic Information System).

Study population

The study population for the present survey consisted of randomly selected people aged five (5) years and above residing in riverine communities of Sokoto State.

Sample size estimation

The total number of individuals enrolled in this study was determined using the following sample size estimation formula reviewed by Sharma [28].

$$\text{Sample Size (n)} = \frac{Z^2 Pq}{d^2}$$

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Where:
n = Desired sample size
Z = Critical value and standard value for the corresponding level of confidence =1.96 at 0.05 level of significance
Prevalence of previous research = 17.8% = 0.178 [31]
q = Probability of failure= 1-P = 1-0.178 = 0.822 and
d = Margin of error or precision = 0.05
Therefore, by substituting the above value into the formula, the desired sample size (n) was:

$$n = \frac{(1.96)^2(0.178)\ (0.822)}{0.05^2}$$

$= 224.8 \approx 225$

Hence, two hundred and twenty-five (225) individuals from each of the four different sample collection centres were recruited for the study.

Questionnaire administration
A questionnaire that was administered for the present study comprised two (2) sections namely; risk factors associated with urinary schistosomiasis and results; and enquiries on the risk factors associated with *S. haematobium* infections [29].

Data collection
Both qualitative and quantitative data were collected from the participants on the field and the participants' samples in the laboratory; the qualitative data include occupations (business, farming, fishing, civil service, hand work, students, housewives, and others), water visit (Yes or No), purpose of water visit (washing, fishing, fetching for domestic use, playing, other purposes), place of urination (nearness/inside freshwater body, bathroom/toilet, bush or anywhere), presence of *S. haematobium* (positive or negative) among the people in the study area while quantitative data consisted of the number of *Schistosoma* eggs (intensity) [30].

Sample collection
Twenty millitter (20 mL) screwed cap, clean, free grease universal sample bottle was offered to each participant to provide with middle to terminal end quantity of urine sample between the h of 10:00 am to 11:30 am every day, a serial number was assigned to each bottle containing the urine sample and serial number corresponded to that of questionnaire carrying the information of the participant. The sample bottles were inserted into the cooler that contained ice bags; all samples were transported to the laboratory for analysis [31].

Detection of *S. haematobium* egg(s) using filtration technique
Ten millilitres (10 mL) of the urine sample was measured and placed onto the filter paper tightened with a handle at the mouth of the filtration flask, then each sample was filtered within five min (5 mins) after switching on the filtration machine, then handle and filtration cup was removed accordingly, forcep was used to take away the filter paper and transferred on to a carbon paper for staining, three drops of Ninhydrin solution were added followed by two drops of Lugo's iodine solution on the filter paper, the filter paper was allowed to dry at room temperature, the entire filter paper was viewed under a microscope using X10 then × 40 objective lenses; several eggs were counted accordingly in the positive samples, the counted eggs were recorded as the number of eggs per 10 mL of urine sample, ≥ 50 eggs/10 mL of urine, indicated heavy infection, 10 to 49 egg/10 mL of urine signified moderate infection and 0 to 10 eggs/10 mL of urine showed low infection [32].

Polymerase Chain Reaction (PCR) analysis of positive samples

DNA extraction
Deoxyribose Nucleic Acid (DNA) was extracted from each urine sample tested positive using QIamp DNA mini kit (QIAGEN, Hilden, Germany), form each urine sample two hundred microliter (200 µL) was placed into a DNA extraction tube (Microtube), 300 µL of lysis buffer was added into the tube, another 500 µL of absolute ethanol was added into the same tube and incubated for the period of 10 min, the solution was transferred into spine column and centrifuged at 8,000 rpm for 1 minute, the supernatant was discarded and the process was repeated for the second time, subsequently, 500 µL of wash buffer (70% ethanol) was added to the sediment and centrifuged at 8,000 rpm for 2 mins, the supernatant was discarded and the process was repeated, another 500 µL of wash buffer was added into the solution and centrifuged at 13,000 rpm for three min, the supernatant was discarded, the solution was transferred into the microtube (Eppendorf tube), 50 µL of molecular grade water was added and centrifuged at 13,000 rpm for 1 minute, the supernatant was discarded and finally, the extracted DNA was stored for the polymerase chain reaction (PCR) [33].

Constitution of the Primers
Forward and reverse primers were constituted according to the manufacturer's instructions. Meanwhile, 100 µM of the forward primer was dissolved in 696.52 µL of buffer, while 100 µM of the reverse primer solution was dissolved in 767.9 µL of buffer solution, the constituted primers were kept at 4°C for further study [34]

PCR Amplification of *S. haematobium*
PCR was conducted to amplify *S. haematobium* at 121 base pair (bp), the PCR of each sample was carried out in a 0.2 mL microfuge tube containing 25 µL reaction mix with the following components; 12.5 µL of Top Taq master mix (Qiagen, USA), 6.5 µL of molecular grade water (Qiagen, USA), 0.5 µL of each 10 µM SH-F and SH-R primers and 5 µL of DNA template. The tubes were transferred to Applied Biosystem (ABS) 9700 Thermocycler and the cycling conditions used were an initial denaturation at 95 °C for 10 mins, followed by 33 cycles of final denaturation at 94 °C for 30 s, annealing at 53 °C for 90 s, and extension at 68 °C for 90 s, followed by a final extension at 60 °C for 5 min and post hold at 8 °C. The PCR amplicons were visualized at 1.5% agarose gel [33]. A list of primers used in the PCR amplification is provided in **Table 1**.

Table 1. Primers used for amplification of *S. haematobium*.

SN	Primers Name	Oligonucleotide Sequence
1	SH-F	5'- GATCTCACCTATCAGACGAAAC-3'
2	SH-R	5'-TCACAACGATACGACCAAC-3'

Agarose gel electrophoresis
Agarose powder of 1g was placed in a conical flask containing 50 mL of buffer, the mixture was homogenized and microwaved at a medium heat temperature, and the solution was allowed to cool to room temperature, 3 µL of ethidium bromide was placed to pre-stained the gel, the solution was transferred into the casting plate with an inserted comb and then allowed to solidify within 30 min, the comb was removed from the casting plate, then the casting plate was placed inside the casting chamber containing 300 mL of buffer solution, the amplified products were subjected to agarose gel electrophoresis with 100 bp ladder used as

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standard. The electrophoresis was carried out at 50 volts for 40 min using a BioRad agarose gel electrophoresis unit. The gel was visualized using a U-V trans-illuminator in a BioRad XRS gel documentation device [35].

Data analysis

Data obtained was subjected to descriptive and inferential statistics, using International Business Machine Corporation-Statistical Package for Social Sciences (IBM-SPSS) software tool version 26.0; occurrence of *S. haematobium* egg(s), and risk factors were calculated accordingly and recorded as percentages while mean egg intensity was calculated by dividing number of eggs counted with number of infected individuals. The presence of *S. haematobium* was considered a dependent variable while risk factors were independent variables; binary logistic regression analysis with an odd ratio was used to identify whether there was significant association and likelihood of getting infection respectively among the individuals, while analysis of variance (ANOVA) was used to determine significant difference between mean intensity of eggs all at $P<0.05$.

RESULTS

Prevalence and intensity of *S. haematobium* among the people from the four (4) locations in the riverine communities of Sokoto State

As shown in **Table 2**; Out of 900 individual samples investigated for the presence of *S. haematobium,* 315 (35.00%) were positive with an average mean egg intensity of 46.28 ± 1.91. Prevalence was observed to be highest [110/225 (48.9%)] in the Wamakko collection centre, followed by Shagari [55/225 (39.11%)], Goronyo [62/225(27.6%)], while participants from Kware had the lowest prevalence [55/225 (24.44%)]. Goronyo participants had a mean intensity of 49.26 ± 2.74, followed by Shagari (48.33 ± 2.11), Kware (46.00 ± 5.64), and Wamakko indicated a mean intensity of 43.11 ± 4.10. However, the intensity of *S. haematobium* in the studied location is not significant (P=0.326).

Table 2. Prevalence and intensity of *S. haematobium* among the people from the four (4) locations in the riverine communities of Sokoto State.

Study Locations	No. Examined	No. Positive	Prevalence (%)	OR (95% CI)	Intensity	P Value
Wamakko	225	110	48.9	3.21 (1.62-3.34)	43.1	0.326
Shagari	225	88	39.1	1.81 (4.18-6.89)	48.3	
Goronyo	225	62	27.6	1.94 (0.26-5.29)	49.3	
Kware	225	55	24.4	1	46.0	
Total	900	315	35.0		46.3	

Prevalence and intensity based on the gender and age of the people in riverine communities of Sokoto State

A higher prevalence of 207/488 (42.4%) was observed among the males, and females had 108/412 (26.2%). Similarly, males indicated the highest mean intensity of 49.60 ± 2.48 than the females (40.02 ± 2.85) and there was a significant difference with regards to the gender of the participants from the study area (P=0.017) (**Table 3**).

According to age groups, those people aged between 46 to 55 and ≥ 56 had the highest prevalence of 7/15(46.7%) and 46/13(46.2%) respectively, followed by those whose ages ranged between 36 to 45 11/32(34.4%), while those aged between 26 to 35 and 5 to 15 recorded lower prevalence of 32/99(32.3%) and 120/363(33.1%) respectively. Significant mean intensity (P=0.011) was noticed to be highest among the aged groups 46 to 55 (70.57 ± 12.089), followed by 26 to 35 and ≥ 56 aged groups (66.65 ± 7.75 and 66.50 ± 16.81 respectively), those individuals aged 36 to 45 (57.20 ± 11.87), aged 16 to 25 had 44.60 ± 2.71, while least mean intensity of 39.66 ± 2.73 was recorded among the individual with age 5 to 15 years old.

Table 3. Prevalence and intensity based on the gender and age of the people in riverine communities of Sokoto State.

Variables	No. Examined	No. Positive	Prevalence (%)	OR (95% CI)	Intensity	P Value
Gender						
Males	488	207	42.4	3.65 (6.71-8.94)	49.6	0.017
Females	412	108	26.2	1	40.0	
Total	900	315	35.0		46.3	
Age						
46 to 55	15	7	46.7	2.11 (0.65-2.11)	70.6	0.011
≥ 56	13	6	46.2	1.56 (1.65-12.36)	66.5	
16 to 25	378	139	36.8	0.56 (2.42-5.98)	44.6	
36 to 45	32	11	34.4	0.17 (11.9–18.91)	57.2	
5 to 15	363	120	33.1	0.11 (0.14-5.41)	39.7	
26 to 35	99	32	32.3	1	66.6	
Total	900	315	35.0		46.3	

Prevalence and intensity of the *S. haematobium* based on the risk factors associated with the infection in the study area

As summarized in Table 4; considering the occupation as a risk factor, a prevalence of 65/112 (57.1%) was observed among the fishermen, followed by Almajiris [14/28 (50.0%)], Farmers [71/154 (46.1%)], Nomadic [3/9 (33.3%)], hand workers [46/143 (32.1%)], businessmen [64/207 (30.9%), Students [44/204 (21.6%)] and housewives had the lower prevalence of 8/43 (18.6%). There was a clear indication that; the average egg intensity of the parasites was high among the Housewives (60.50 ± 12.92), followed by Almajiris (57.14 ± 5.77), fishermen (53.72 ± 4.99), hand workers (49.72 ± 5.67), farmers (46.00 ± 4.26), students (45.18 ± 2.55), and businessmen (34.54 ± 3.69) and nomadic recorded the lowest mean intensity of 21.00 ± 9.85. A significant difference was observed (P=0.000).

Participants who used any available water source for domestic cores had the highest prevalence of 10/10(100.0%), followed by those individuals using River/Dam/Lake water [112/244(45.9%)] then well [115/342 (33.6%)], borehole [50/178 (28.1%)], and participants with tap as water source had the lower prevalence of 28/126 (22.2%). Mean intensities of 53.40 ± 17.91, 52.41 ± 3.04 and 50.50 ± 8.12 were observed among individuals who used any available, rivers/dams/lakes and tap water sources respectively. In contrast, those that used boreholes and wells water were observed with 42.52 ± 5.01 and 40.41 ± 2.71 respectively. There was no significant difference (P=0.197)

People who visited freshwater bodies had the highest prevalence of 305/896 (37.72%) while those who were not visited, recorded prevalence of 10/94 (10.63%). The highest mean intensity of 58.70 ± 10.81 was recorded among Individuals who visited freshwater bodies while those who had not visited the water bodies had a mean egg intensity of 45.88 ± 1.940. Significant difference was observed (P=0.009). Based on purposes of freshwater body visit, individuals visited freshwater body for irrigation had the highest prevalence of 28/53 (52.8%) followed by fishing [37/75 (49.3%)], multiple purposes [103/211 (48.8%)], swimming [46/107 (42.9%)], washing [22/56 (39.3%)], watering animals [18/46 (39.1%)], passing [35/164 (21.3%,)], fetching [14/68 (20.6%), watching [2/15 (13.3%)], where those with other purpose had the least prevalence of 10/105 (09.5%). The current survey observed that people who were washing, and fetching recorded the highest mean egg intensity of 65.79 ± 9.03, and 55.44 ± 11.53 respectively, followed by swimming, fishing, and watering animals (49.93 ± 8.56, 48.53 ± 5.45, 44.22 ± 5.19 respectively), then other

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purposes, watching, and passing with 38.67 ± 16.17, 37.95 ± 4.64, and 36.69 ± 4.93 respectively. No significant difference (P=0.059).It was reported that people who were very close to the freshwater bodies were highly infected [207/499 (41.5%)] followed by those far away from the water bodies [108/401 (26.9%)]. Also, the higher mean intensity was high among the participants that are very close to water bodies (48.41 ± 2.59) than those who were far away from water bodies (42.27 ± 2.50), however, there was no significant difference (P=0.126). According to a place where the respondents urinated, individuals urinated anywhere were mostly infected [28/40 (70.0%)], followed by inside/near water bodies [94/49 (63.1%)], bush [93/273 (34.1%)], and bathroom/toilet had lower prevalence of 100/438 (22.8%). Average intensity was significantly high (P=0.000) people urinated inside/near freshwater bodies (57.19 ± 4.00), followed by bush (49.37 ± 2.84), bathroom/toilet (40.48 ± 3.31) while those who urinated anywhere had the lowest intensity of 21.77 ± 3.80.

Prevalence of 180/441 (40.8%) and 135/459 (29.4%) was observed among the participants that do not attend and attended formal schools respectively. Also, no significant (0.782) mean intensity of 48.43 ± 3.15 and 44.69 ± 2.37 was confirmed among those who did not attend and attended formal schools respectively. Illiterates were highly infected [185/441 (41.9%)], followed by those at the primary level of education [75/241 (31.1%)], secondary [53/169 (31.4%)], and those at tertiary recorded smallest infection rate of 2/49 (4.1%). Similarly, the Illiterate recorded the highest mean egg intensity of 55.50 ± 6.50 followed by those at secondary (54.28±5.29), tertiary (45.23±2.35), and primary had the least intensity of 42.96±3.97. There was no significant difference (P=0.523).

Prevalence [62/129 (48.06%)] was the highest among the individuals with no information on bilharziasis where 252/771 (32.8%) infected persons had no information. More so, significant (P=0.001) mean intensities of 59.32 ± 3.59 and 43.09 ± 2.17 were observed among the participants with no information and those with the information respectively. With regards to knowledge of the causes of bilharziasis, it was observed that, individuals who didn't know the cause recorded the highest prevalence of 194/291 (65.3%) followed who said others [108/430 (25.12%)], snails [10/97 (9.7%)] and *S. haematobium* had the least prevalence of 3/82 (3.7%). The intensity was high among the individuals who didn't know the cause of the disease (50.88 ± 8.38), followed by those who said it was snail (49.10±8.38), *S. haematobium* (41.33±3.76), while others had least intensity of 37.91±3.22. However, there was no significant difference (P=0.464).

Participants who didn't know the cause of bloody urine had the highest prevalence of 298/814(36.6%) while those who knew the cause had a prevalence of 19.8%. According to the knowledge of the causes of bloody urine. The intensity was also high among the respondents with knowledge of the causes of bloody urine (51.59 ± 4.46) whereas those whose don't know the cause of bloody urine recorded an intensity of 45.98 ±2.00 with no significant difference (P=0.508). Persons who received treatment before had the highest prevalence of 172/255 (67.5%), while those who were not treated before had a prevalence of 143/645 (22.2%). association was not significant (P=0.261). Although, the intensity was significant (P=0.002) according to the history of receiving treatment among the participants, those that have received treatment recorded the highest intensity of 51.74 ± 2.589, and those that were not received treatment before had the average intensity of 39.81 ± 2.74.

Molecular study of the infected sample using PCR

Table 5 indicated that; Out of 315 positive individuals analyzed using PCR, 276/315 (87.6%) were confirmed positive. Wamakko participants showed the highest prevalence of 107/110 (97.3%), followed by Shagari [84/88 (95.5%)], Kware [42/55 (76.4%)] and lastly Goronyo [43/62 (69.4%)]. **Figs 2** and **3** showed *S. haematobium* at 121 bp using 1.5% agarose gel. Males had the highest prevalence of 197/207 (95.2%) than females [79/108 (73.2%)]. People aged between 5 to 15 years old had the highest prevalence of 118/120 (98.3%), followed by 16 to 25 years old [116/139 (83.45%)], ≥ 56 years old [5/6 (83.3%), 26 to 35 years old [25/30 (78.1%)], 36 to 45 years old [8/11 (72.7%)], and 46 to 55 years old [4/7 (57.1%)].

Based on the participants' occupations; fishermen had the highest prevalence of 64/65 (98.5%), followed by farmers 68/71 (95.8%), hand workers [68/71 (89.1%)], students [38/44 (86.4%) Almajiris [12/14 (85.7%) Business [46/64 (71.9%)]. Nomadic and housewives had a lower prevalence of 2/3 (66.7%) and 5/8 (62.5%), respectively. Individuals with river/lake/dam as their source were confirmed with the highest prevalence of 111/112 (99.1%), followed by available [9/10 (90.0%)], well [102/115 (88.7%)], while borehole and tap recorded 38/50 (76.0%) and 16/28 (57.1%) respectively. People with and without a history of freshwater body visits had a prevalence of 274/305 (89.8%) and 2/10 (20.0%) respectively. Those who visited the freshwater for Swimming, Fishing, Irrigation, or Multiple purposes recorded prevalences of 45/46 (97.8%), 36/37 (97.3%), 27/28 (96.4%), or 98/103 (95.2%) respectively, while passing, washing, watering animals, other purposes and watching had 31/35 (88.6%), 17/22 (62.9%), 11/18 (61.1%), 4/10 (40.0%) and 0/2 (0.0%) respectively.

Individuals who are very close and far away from the water bodies had a prevalence of 204/207 (98.6%) and 72/108 (66.7%) respectively. People who urinated inside or near freshwater bodies were confirmed with the highest prevalence of 93/94 (98.9%), followed by inside bathrooms or toilets [84/100 (84.0%)], anywhere [23/28 (82.14%)], while bush recorded the lowest prevalence of 76/93 (81.7%). A significant association was observed (P=0.000). Considering literacy as a risk factor; participants who did not attend formal school were confirmed with the significant (P=0.000), highest prevalence of 173/180 (96.1%) and 103/135 (76.3%) respectively. Those who did not attend any level of education were confirmed with the highest prevalence 178/185 (96.0%), followed by primary 69/75 (92.0%), secondary 69/75 (52.8%), and tertiary level had the least prevalence of 1/2 (50.0%). Also, there was a significant association (P=0.006).

Participants who didn't have information about the disease were confirmed with the highest prevalence of 227/252 (90.1%) and 49/62 (79.0%) confirmed for those with the information. However, no significant association (P=0.775). People responded to the bloody urine was caused by spiritual were confirmed with the significant (P=0.002) highest prevalence of 179/194 (92.3%) followed by others [94/108 (87.0%)], snail [3/10 (30.0%)], while 0/3 (0.0%) was confirmed for the *S. haematobium* respondents. Participants with and without knowledge of the causes of bloody urine were confirmed with the significant (P=0.000) prevalence of 8/17 (89.9%) and 268/298 (89.9%) respectively. Also, people who had treatment before were confirmed with the significant (0.001) highest prevalence of 164/172 (95.4%), while those who did not receive treatment before were confirmed with 112/143 (78.3%) prevalence.

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Table 4. Prevalence and intensity of the *S. haematobium* based on the risk factors associated with the infection in the study area.

Variables	No. Examined	No. Positive	Prevalence (%)	OR (95% CI)	Intensity	P-Value
Occupation						
Fishermen	112	65	57.1	5.19 (1.86-15.24)	53.7	0.000
Almajiris	28	14	50.0	4.11(12.61-25.01)	57.1	
Farmers	154	71	46.1	2.87 (0.28-1.76)	46.0	
Nomadic	9	3	33.3	0.92 (0.16-13.42)	21.0	
Hand workers	143	46	32.2	0.74 (0.23-21.56)	49.7	
Business people	207	64	30.9	1	34.5	
Students	204	44	21.6	0.43 (0.21-2.13)	45.2	
H/Wives	43	8	18.6	1	60.5	
Total	900	315	35.0		46.3	
Water Source						
Available	10	10	100.0	1.04 (0.68-1.56)	53.4	0.197
River	244	112	45.9	1.51 (1.43-12.80)	52.4	
Well	342	115	33.6	0.34 (0.61-8.14)	40.4	
Borehole	178	50	28.1	0.19 (5.56-11.37)	42.5	
Tap	126	28	22.2	1	50.5	
Total	900	315	35.0		46.3	
Waterbody Visit						
Yes	896	305	37.7	5.16 (1.86-11.91)	58.7	0.009
No	94	10	10.6	1	45.9	
Total	900	315	35.0		46.3	
Purpose of the Visit						
Irrigation	53	28	52.8	12.52 (1.06-1.96)	40.7	0.059
Fishing	75	37	49.3	13.3 3(1.98-2.61)	48.5	
M. Purpose	211	103	48.8	0.51 (0.32-2.07)	47.1	
Swimming	107	46	42.9	0.18 (0.72-21.93)	49.9	
Washing	56	22	39.3	0.87 (0.53-5.91)	65.8	
W/Animals	46	18	39.1	0.79 (0.62-9.63)	44.2	
Passing	164	35	21.3	0.43 (0.71-15.65)	36.7	
Fetching	68	14	20.6	0.87 (0.87-2.61)	55.4	
Watching	15	2	13.3	0.79 (0.47-0.81)	37.1	
Other Purpose	105	10	09.5	1	38.7	
Total	900	315	35.0		46.3	
Water Closeness						
Very Close	499	207	41.5	3.54 (1.43-8.46)	48.4	0.126
Far away	401	108	26.9	1	42.3	
Total	900	315	35.0		46.3	
Urination Place						
Anywhere	40	28	70.0	0.51 (0.65-29.11)	21.8	0.000
Near/Inside Water	149	94	63.1	1.02 (0.27-0.56)	57.2	
Bush	273	93	34.1	0.72 (0.43-0.61)	49.4	
Bathroom/Toilet	438	100	22.8	1	40.5	
Total	900	315	35.0		46.3	
School Attendance						
No	441	180	40.8	14.36 (1.97-10.54)	44.7	0.782
Yes	459	135	29.4	1	48.4	
Total	900	315	35.0		46.3	
Education Level						
None	441	185	41.9	0.518 (0.26-1.47)	55.5	0.523
Primary	241	75	31.1	1.26 (4.25-8.65)	42.9	
Secondary	169	53	31.4	0.92 (2.18-7.12)	54.3	
Tertiary	49	2	4.1	1	45.2	
Total	900	315	35.0		46.3	
Bilharz Information						
No	129	62	48.1	0.28 (0.54-14.43)	59.3	0.001
Yes	771	252	32.8	1	43.1	
Total	900	315	35.0		46.3	
Causes						
Don't Know	291	194	65.3	0.69 (0.84-30.34)	50.9	0.464
Others	430	108	25.1	0.32 (0.62-16.22)	37.9	
Snail	97	10	9.7	0.41 (0.12-2.63)	49.1	
S. haematobium	82	3	3.7	1	41.3	
Total	900	315	35.00		46.3	
Bloody Urine Cause						
No	814	298	36.6	1.32 (5.61-8.21)	45.9	0.508
Yes	86	17	19.8	1	51.6	
Total	900	315	35.00		46.3	
History of Treatment						
Yes	255	172	67.5	0.60 (0.25-1.46)	51.7	0.002
No	645	143	22.2	1	39.8	
Total	900	315	35.00		46.3	

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Table 5. Molecular study of the infected sample using PCR.

Variables	No. Examined	No. Positive	Prevalence (%)	OR (95% CI)
Locations				
Wamakko	110	107	97.3	4.16 (1.13-8.58)
Shagari	88	84	95.5	3.27 (2.25-8.54)
Kware	55	42	76.4	0.21 (0.77-4.12)
Goronyo	62	43	69.4	1
Total	315	276	87.6	
GENDER				
Males	207	197	95.2	1.65 (1.65-9.94)
Females	108	79	73.2	1
Total	315	276	87.6	
Age				
5 to 15	120	118	98.3	3.49 (1.08-3.95)
16 to 25	139	116	83.5	1.04 (0.63-0.81)
≥ 56	6	5	83.3	0.87 (0.75-0.96)
26 to 35	32	25	78.1	0.53 (0.62-1.98)
36 to 45	11	8	72.7	1.61 (0.47-0.93)
46 to 55	7	4	57.1	1
Total	315	276	87.6	
RISK FACTORS				
Occupation				
Fishermen	65	64	98.5	12.32 (2.54-3.91)
Farmers	71	68	95.8	1.05 (0.79-0.92)
Handwork	46	41	89.1	0.54 (0.87-0.93)
Students	44	38	86.4	0.61 (0.87-10.83)
Almajiris	14	12	85.7	0.32 (0.61-0.82)
Business	64	46	71.9	0.21 (0.76-0.89)
Nomadic	3	2	66.7	0.18 (0.43-0.98)
Housewives	8	5	62.5	1
Water Source				
River	112	111	99.1	3.16 (1.15-2.36)
Available	10	9	90.0	1.18 (2.24-6.76)
Well	115	102	88.7	0.79 (0.73-0.95)
Borehole	50	38	76.0	0.11 (0.18-21.65)
Tap	28	16	57.1	1
Total	315	276	87.6	
Waterbody Visit				
Yes	305	274	89.8	16 (4.36-10.64)
No	10	2	20.0	1
Total	315	276	87.6	
Purpose of Visit				
Swimming	46	45	97.8	1.71 (1.81-14.17)
Fishing	37	36	97.3	1.65 (1.41-3.82)
Irrigation	28	27	96.4	0.98 (0.71-0.91)
Multiple Purposes	103	98	95.2	2.87 (1.12-10.98)
Passing	35	31	88.6	0.93 (0.91-2.17)
Washing	22	17	62.9	0.76 (0.25-0.91)
Watering Animals	18	11	61.1	0.64 (0.87-9.45)
Fetching	14	7	50.0	0.36 (0.27-1.79)
Other Purpose	10	4	40.0	1
Watching	2	0	0.0	
Total	315	276	87.6	
Nearness to the Water				
Very Close	207	204	98.6	2.45 (1.11- 6.25)
Far away	108	72	66.7	1
Total	315	276	87.6	
Urination Place				
Near/Inside Water Body	94	93	98.9	4.01 (0.71-1.98)
Bathroom/Toilet	100	84	84.0	0.59 (0.03-9.07)
Anywhere	28	23	82.1	0.36 (0.11-12.15)
Bush	93	76	81.7	1
Formal School Attendance				
No	180	173	96.1	2.61 (1.25-4.91)
Yes	135	103	76.3	1
Total	315	276	35.0	
Level of Formal Education				
None	185	178	96.2	2.85 (1.96-3.65)
Primary	75	69	92.0	0.93 (0.39-6.91.)
Secondary	53	28	52.8	0.67(0.63-2.31)
Tertiary	2	1	50.0	1
Total	315	276	35.0	
About Bilharziasis				
No	252	227	90.1	1.64 (0.56-0.59)
Yes	62	49	79.0	1
Total	315	276	35.0	
Causes of Bilharziasis				
Spirituals	194	179	92.3	8.13 (0.23-6.93)
Others	108	94	87.0	1.14 (0.14-2.13)
Snail	10	3	30.0	1
S. haematobium	3	0	0.0	
Total	315	276	35.0	
Cause of Bloody Urine				
No	298	268	89.9	0.38 (0.29-16.36)
Yes	17	8	47.1	1
Total	315	276	35.0	
History of Treatment				
Yes	172	164	95.4	0.51 (0.13-0.92)
No	143	112	78.3	1
Total	315	276	35.0	

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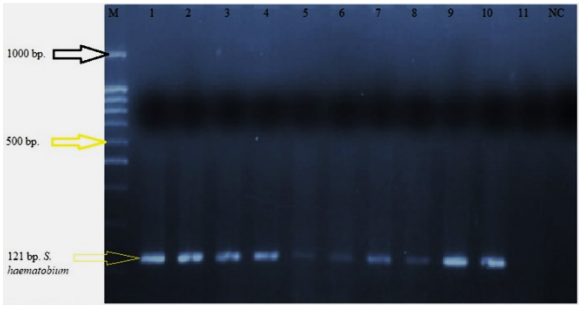

Fig. 2. 1.5% agarose gel electrophoresis analysis for the detection of *S. haematobium* among the people in riverine communities of Sokoto State, Sokoto. Key: M molecular ladder. NC=negative control, positive samples= 1 to 10, Negative sample = 11.

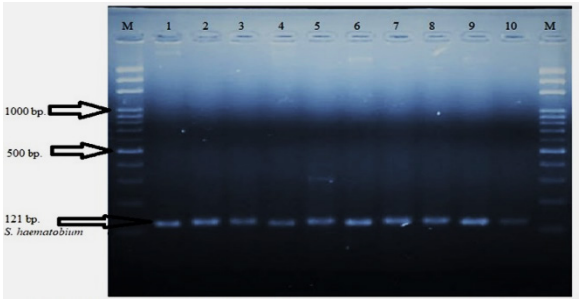

Fig. 3. 1.5% agarose gel electrophoresis analysis for the detection of *S. haematobium* among the people in riverine communities of Sokoto state. Key: M molecular ladder. NC=negative control, positive samples= 1 to 10.

DISCUSSION

Schistosomiasis caused by *S. haematobium* remained one of the major public health issues in tropical and subtropical areas [36, 37, 38]. Due to the significant connection between the infection rate and place of living, gender, age, risk factors as well as clinical manifestations, the importance of epidemiological studies on the prevalence of *S. haematobium* based on those factors is over-emphasized to look for the numerous ways to address the challenges associated with the disease emergence and reemerging [39-41].

Subsequently, this epidemiological study conducted in riverine communities of Sokoto State showed that 35.0% of the participants were infected with a mean egg intensity of 46.28 $\pm$ 1.91. This prevalence was confirmed to be 87.6% specific using the PRC test. The observed prevalence was lower than the earlier infection rate reported from some communities of Sokoto State. For instance, Singh and Muddasiru [42] reported 60.8% were infected in riverine communities of Wamakko, 61.8% were infected in Sokoto South [43]; 48.0% of pupils were also infected in Wamakko [45], 60.8% were reported infected in Kware and Sokoto south [29].

The overall prevalence observed presently was in the same range as the reports of Iduha and Bwari [46], who observed that 37.0% were infected in Goronyo and Taloka Districts, Muhammad and Bala [47], also reported 32.8% were infected in riverine communities of Wamakko, additionally, 38.3% were reported from Wamakko town [25, and 32.3% were found in settlement of Goronyo [48]. However, the report was higher than several reports in the states as Yunusa *et al.,* [49], reported 17.2% were infected in Kware, 21.3% were reported from Salami [50], and 12.1% were reported from Wamakko [51]. The infection rate was highly (48.9%) significantly associated with OR (3.21), 95% CI 1.62-3.34 in Wamakko and Shagari (31.9%) OR (1.18) 95% CI 1.18-6.89 collection centre. These could be attributed to their degree of dependence on freshwater bodies for their everyday supplements than others [52]. The majority of the people from the Wamakko and Shagari collection centres engaged themselves in occupational activities such as fishing, rice farming, washing vehicles, swimming as well and fetching water from the rivers for domestic use. It was reported that the prevalence of schistosomiasis is significantly associated with the population's dependence on untreated water bodies for their everyday activities [53].

Males were highly (42.4%) significantly associated [OR (1.18) 95% CI: 6.71-8.94] and moderate intensity (49.6) with a significant difference (P= 0.017). This outcome is consistent with prior research [54], however, opposed to studies that indicated a higher frequency among females [55]. Such variations may be attributed to the various cultural and religious practices that permit males to explore freshwater bodies than females in the study areas. The consequently higher prevalence of the schistosome parasite in males may be attributed to traditional values and the sociocultural practices, and behaviours of the people living in the studied location [56]. Since the bulk of residents in Sokoto State's riverine areas are Hausa Fulani and Muslims, women are generally well-safeguarded and have limited freedom of movement compared to their male counterparts and therefore, females have less contact with bodies of water. Additionally, swimming naked outside is socially inappropriate for both young and older ladies because girls mature earlier than their male counterparts [57].

The age-specific prevalence of 46.2% was observed among the old age group ($\geq$56) with significant association [OR (1.56) 95% CI: 1.65-12.36] with heaviest (66.5) significant (P=0.011) mean egg intensity. This group tends to engage in regular water-contact activities such as irrigating, fishing, and swimming in freshwater areas in addition to the weak immunity due to old age, which are probably risk factors for the increased infection rate. Other age groups engage in similar water contact behaviour, but they attend formal school more frequently and have access to information on infection prevention. These reports concur with those made by Babatunde *et al.* [58], who noted an increase in the prevalence of infection among those between the ages of 40 and 60.

In contrast, [59] asserted that the trend of infection prevalence rate among older age groups was gradually declining. Additionally, it was discovered that elderly individuals with lower worm burdens may have developed immunity, which is known to happen throughout infection. This is consistent with studies on egg decreases in other *S. haematobium* endemic regions [60]. The highest infection rate (57.1%) of the disease among fishermen with a significantly (P=0.000) heavy average intensity (53.72) may be related to occupational activities that required frequent use of and contact with freshwater sources. This study was comparable to research done in Nigeria, where frequent water contact patterns were found to be responsible for the *S. haematobium's* frequency and distribution [61]. It also echoes findings made by Nkengazong *et al.* [62], who demonstrated that individuals engaged in occupations like fishing, which require interaction with freshwater bodies, maintained a high level of infection in southwest Cameroon. The highest infection rate (99.1%) was confirmed among people who depend largely on rivers, lakes and dams for their water source. This can be explained by the natural preference that snail vectors

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have for slow-moving rivers or still bodies of water [63, 25]. Even though providing safe drinking water from a well tap, borehole, etc. has been advised for the management of schistosomiasis [64], an infection rate was also seen among those who drank water from boreholes, wells, and other sources. This suggests that other contact activities with contaminated water in the vicinity may have contributed to the infection.

People who come into contact with infested freshwater sources that act as schistosome parasite breeding grounds are more likely to contract *S. haematobium* infection; this may be because people in most rural areas may have unintentionally come into contact with the parasites through water sources that accidentally penetrated their skin and later became infected [16], the difference in infection prevalence among the participants could be explained by the fact that residents in Sokoto State's riverine regions mostly rely on rivers, dams, ponds, and wells for their water supply, which puts them at risk for contracting the illness. Few residents of the urban riverine districts rely exclusively on wells for their daily water supply; some of them depend on other sources.

The riverine communities of Sokoto state are rural, and the majority of the villages rely on rivers and lakes for their water needs, including bathing, swimming, fishing, and other domestic purposes. This observation is consistent with the findings of Senghor *et al.* [65], in Niakhar, Senegal who observed that water bodies that served as natural water supplies were not identified only as a source of water in the study area, but also provided habitats for the intermediate hosts and the schistosome parasites. This study has shown that a highly significant prevalence rate of the infection occurs in those whose residences were located very close to the water bodies. This could be because the freshwater bodies have an occupational and tourist attraction that encourages them to engage in more water-contact activities such as farming/irrigating, playing, washing, fetching, drinking, and swimming etc. than people who live far from the freshwater bodies.

According to a CDC report from 2008, the majority of cases of schistosomiasis disease are contracted through the skin, although drinking contaminated water with cercariae may cause cercariae to penetrate the mucous membrane and cause the infection, they added that swimming, bathing, or working in contaminated water offer the greatest risk of infection. People who live adjacent to water bodies are more likely to engage in water-related activities in Sokoto state's riverine zones because they frequently visit freshwater for drinking, cooking, and other uses. Concerning formal school attendance and the level of education of the people in the riverine communities of Sokoto, it has been shown that illiterate people were highly infected. This predominance may be related to personal practices such as inadequate hygiene and sanitation, socioeconomic behaviour, and occupations that require them to come into touch with water bodies more frequently than the area's literate population. These findings are in line with an established report [59], that the increased disparities in prevalence rates could be attributed to a variety of variables, including the literateness of the population, activities that require frequent contact with water, the degree of exposure to contagious schistosome cercariae, and the unique ecological traits of various regions.

CONCLUSION

The comprehensive study across four riverine communities in Sokoto State, Nigeria, uncovers the pervasive challenge of *Schistosoma haematobium* infections, affecting a substantial portion of the population, with a prevalence rate of 35%. The research highlights significant variances in prevalence and intensity among different locations, gender, age groups, and other risk factors, emphasizing the critical need for targeted interventions. The highest prevalence was observed in Wamakko, signalling a pressing health concern. Gender and age emerged as significant determinants of infection rates and intensity, with males and older age groups more affected, suggesting a correlation between these demographics and exposure to risk factors. Occupationally, fishermen and individuals with direct contact with riverine environments face heightened risks. Molecular analysis confirmed a majority of the infections, underscoring the utility of PCR in enhancing diagnostic accuracy. This study accentuates the urgent requirement for comprehensive health education, improved sanitation, and access to safe water to mitigate the transmission and impact of schistosomiasis in affected communities.

RECOMMENDATIONS

Enough facilities should be provided for conducting both sensitivity and specificity analysis of the samples because sensitivity may result in a false positive sample since it is not 100% effective, also government and non-governmental organizations should always use findings obtained from epidemiological surveys as a guide for the treatment, prevention and control of *S. haematobium* and other form NTDs.

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COMPETING INTEREST

The authors declared that no competing interest.

ETHICAL CONSIDERATION

The present research considered all the ethics by the ethical research committee of Sokoto State Ministry of Health; hence, an introduction letter was collected from the Head of Department, Department of Animal and Environmental Biology, Kebbi State University of Science and Technology Aleiro; the letter was taken to the Ethical Research Committee, Sokoto State Ministry of Health, to obtained ethical clearance letter for conducting the research; the ethical clearance was approved with the reference number SKHREC/10/2023 [41].

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used SPSS order to make data analysis Quillbot inc. to paraphrase the statements, Grammarly to check the correctness of the grammar and avoid spelling mistakes, Turnitin to check the similarity index, Painter to make the plates clearer and Mendeley to make citations and bibliography. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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