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Systematic Review

Genetic Diversity of Schistosoma Haematobium in Sub-Saharan Africa: A Systematic Review

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Abstract: Urinary schistosomiasis caused by the parasite *Schistosoma haematobium* is the most common form of schistosomiasis. This parasite has a high potential for genetic exchange within parasite populations giving rise to the genetic diversity that is important for its survival. Genetic differences may lead to some parasite strains being more immunogenic which may have a negative impact on the management and control of schistosomiasis. Therefore, understanding these genetic differences in the parasite may lead to better management of the disease. A literature search was done on PubMed, African Journals Online and Google scholar using predefined search terms such as urinary schistosomiasis, *S. haematobium*, genetic diversity in sub-Saharan Africa in combination with Boolean operators (AND, OR). The search included studies published from 2000 to 2020 that emphasised on genetic diversity of *Schistosoma haematobium* in sub-Saharan Africa. Sixteen studies from 18 sub-Saharan African countries that met the inclusion criteria were selected. Most studies conducted in these countries showed a high genetic diversity of *Schistosoma haematobium* studies with microsatellite markers being the most commonly used method for genetic diversity determination. Fisher's exact test showed that the distribution of genetic diversity in sub-Saharan African regions was not statistically significant (p=0.768). The highest number of studies on genetic diversity of *Schistosoma haematobium* were conducted in West Africa with Nigeria and Zimbabwe in Southern Africa conducting the most studies, 4/36 (11%) each. Results obtained show the need for continued monitoring of genetic variations in *Schistosoma haematobium* in sub- Saharan Africa. This will aid in understanding the epidemiology of the disease, advancing novel treatment and vaccine strategies.

Keywords: Urinary schistosomiasis, *Schistosoma haematobium*, sub-Saharan Africa, Genetic Diversity

1. Introduction

Schistosomiasis is the most widely distributed neglected tropical disease (NTD) and second to malaria in terms of human morbidity, mortality and socio-economic importance [1–3]. It remains a public health problem in many parts of the world despite having measures to combat the global prevalence. Worldwide, about 250 million people in 78 countries are infected, of which 42 countries are in Africa [4]. Infection rates in sub-Saharan Africa (SSA) account for over 85% of a population that makes up 13% of the world's

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population [5,6]. The World Health Organization (WHO) estimated that 243 million people with 226 million being in Africa, including 111.2 million school-aged children and 92.5 million adults needed preventive chemotherapy [1]. The target was to treat a minimum of 75% and up to 100% school-aged children at risk of morbidity by 2010. This target was not achieved, and new goals were set for the year 2020 which were 100% geographic coverage, 75% national coverage and <5% prevalence of heavy infection [4]. While other countries such as Japan, Tunisia and Morocco have successfully eliminated this disease, it is still endemic in various SSA communities, and the mortality and morbidity associated with it cannot be overemphasised [7].

Trematode worms of the genus Schistosoma cause the disease. Species of Schistosoma that are known to cause human infection include Schistosoma haematobium, Schistosoma mansoni, Schistosoma japonicum, Schistosoma guineensis, Schistosoma intercalatum and Schistosoma mekongi. However, the most common disease-causing organisms are S. haematobium, S. mansoni, and S. japonicum [8,9]. Of the three Schistosoma species, S. haematobium is the most widely distributed schistosome and is responsible for causing human urogenital schistosomiasis. It accounts for over 112 million cases in SSA and the Middle East. These S. haematobium cases represent almost half the total worldwide cases and more than double the estimated figure for S. mansoni [2,10,11].

Schistosomes have a potential for genetic exchange within parasite populations, and this genetic diversity is thought to be vital for their ability to survive the pressures within their environment. The genetic diversity of pathogens such as parasites can be affected by various factors such as environmental influence, host immunity and large scale administration of anthelminthic drugs such as praziquantel. This diversity might impact the management and control of urogenital schistosomiasis. It is also believed to have a major positive influence on parasite-related characteristics, including dynamics of transmission, host-parasite interaction, infectivity, and virulence [11]. It may also be important in determining the disease's clinical outcome, ranging from mild symptoms to severe damage of the kidneys and/or bladder [14]. The genetic diversity of parasites is an important factor that determines their potential in producing harmful effects among human or host populations they parasitize. Diversity of the parasites may play an important role in the pathology of schistosomiasis, which may result in different clinical outcomes and also in some parasites being more immunogenic than others [14]. Studying the genetic diversity of schistosome parasites allows linking some genotypes with disease prevalence, and this can then be used to formulate effective control measures.

Praziquantel (PZQ) remains the drug of choice for schistosomiasis treatment and morbidity control [17,18]. It can be administered easily at a standard oral dose of 40mg/kg and improves the health and well-being of infected people. Large scale administration or mass drug administration (MDA) of PZQ is used to alleviate the burden of schistosomiasis in many sub-Saharan African countries. Mass drug administration is even more focused on School-aged children because these are thought to be the ones most likely to be infected due, in part, to their water-contact patterns [19]. This is likely, though, to place strong and novel selective pressures on the parasites, which may impact their population structure and genetic diversity [20].

In SSA, molecular methods such as mitochondrial DNA barcoding, microsatellite analysis and Randomly Amplified Polymorphic DNA [RAPD] are being used for the determination of genetic diversity and population structure of schistosomes [11,20,21]. The RAPD-PCR has been used as a valuable tool to explore the genetic diversity in schistosomes, especially in snails. The primers can screen a wide range of loci across the genome with low DNA yields and limited available sequence data [22]. Mitochondrial DNA barcoding has been widely used to detect any sequence variation in the mitochondrial genome that may occur over time. Such methods have largely benefited from the knowledge of the complete mitochondrial genome of the *S. haematobium* [20]. Meanwhile, microsatellite markers are highly variable markers that have been widely used in the schistosomiasis research community. These markers may be used to detect any genetic drifts, gene flow

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and estimate any changes mediated by mass treatment. Molecular methods are therefore able to elucidate the epidemiology and evolution of schistosomiasis and also monitor and evaluate the impact of progressing control programs. They are, however, expensive and labour intensive [21]. In contrast, routine diagnostic methods such as urine filtration methods are less expensive but they continue to revolve around egg detection, which sometimes proves difficult and less sensitive [23]. The current review, therefore, aimed at determining the genetic diversity and molecular epidemiology of *S. haematobium* in SSA countries and the different methods used to determine its genetic diversity. The current review contributes to the knowledge that may influence policymakers in SSA concerning treatment, control and elimination of urinary schistosomiasis in SSA.

2. Results

2.1. Search Results

All articles included in the current study were research articles conducted within SSA countries. Initially, a total of 878 records were identified through database searching with an additional four articles added from snowballing, making a total of 882 articles. However, only 16 articles were eligible for the study, and others were not eligible. The process followed is shown in the PRISMA flow diagram below (Figure 1).

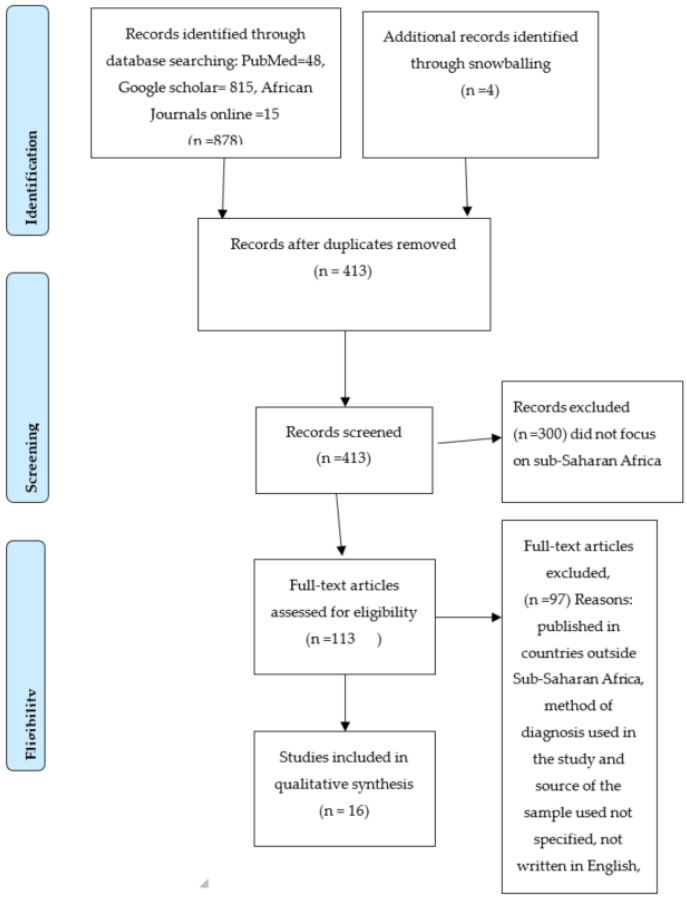

Figure 1: Preferred Reporting Systems for Systematic and Meta-Analyses (PRISMA) Flow Diagram for articles included in the current review

2.2. Genetic Diversity of S. haematobium

The majority of the countries showed high genetic diversity of S. haematobium with 18/32 (56%) studies showing a high genetic diversity followed by low 10/32 (31%) and

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moderate 4/32 (13%) in various regions of SSA (Figure 2). However, fisher's exact test showed that the distribution of genetic diversity in SSA regions was not statistically significant (p = 0.768). Fisher's exact test was also used to determine the association between genetic diversity and regions within sub-Saharan Africa. However, there was no statistically significant association between region and genetic diversity (p=0.84).

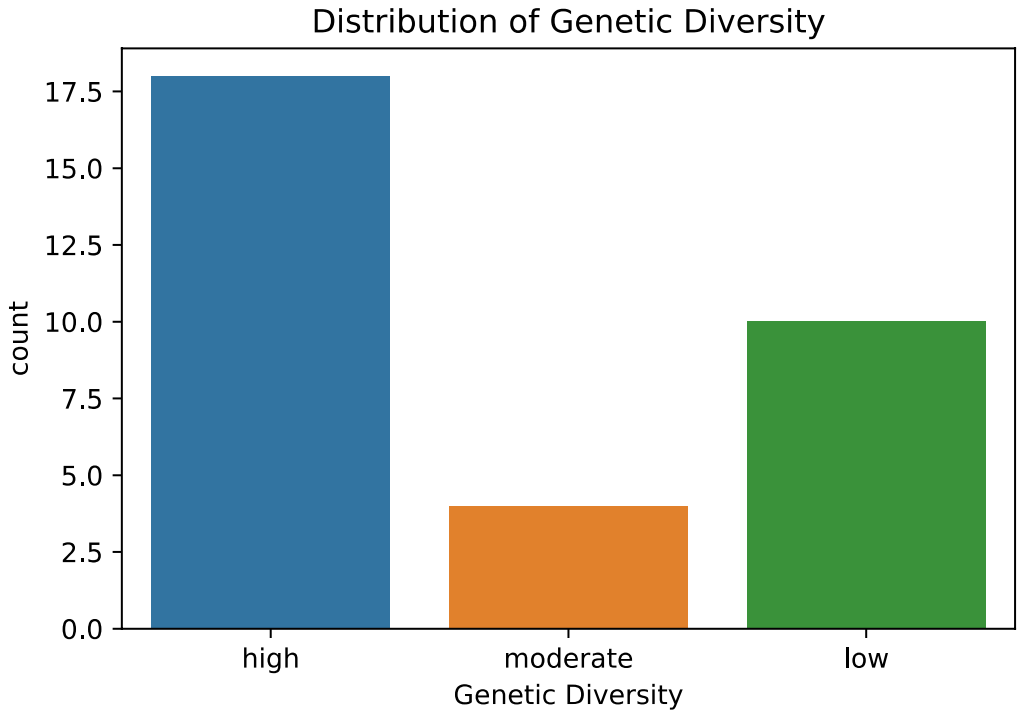

Figure 2: Showing the genetic diversity of S. haematobium from studies conducted in SSA countries. Most of them reported a high genetic diversity

2.3. Methods of genetic diversity determination

The results in the current study show that various methods were used to determine the genetic diversity and molecular epidemiology of S. haematobium and that the most commonly used method was microsatellite markers followed by DNA barcoding. The following was the distribution of the methods used: microsatellites were used in 20/36(56%) studies, DNA barcoding in 9 (25%) studies, RAPD in 6 (16%) studies, mitochondrial cox1 DNA in 9 (25%) studies, nuclear ITS2 in 1 (3%) study.

2.4. Characteristics of studies included

The reviewed articles were published between 2000 and 2017 from 18 SSA countries. The year 2012 has the most studies 11(30%) followed by 2013, 8(22%). Figure 3, shows the geographical location of all the 18 SSA countries in which studies the genetic diversity of *S. haematobium* was determined. Table 1, provides a summary of all the included studies; author/year of publication, country of study, methods of genetic diversity determination, study objectives and the major findings of the study.

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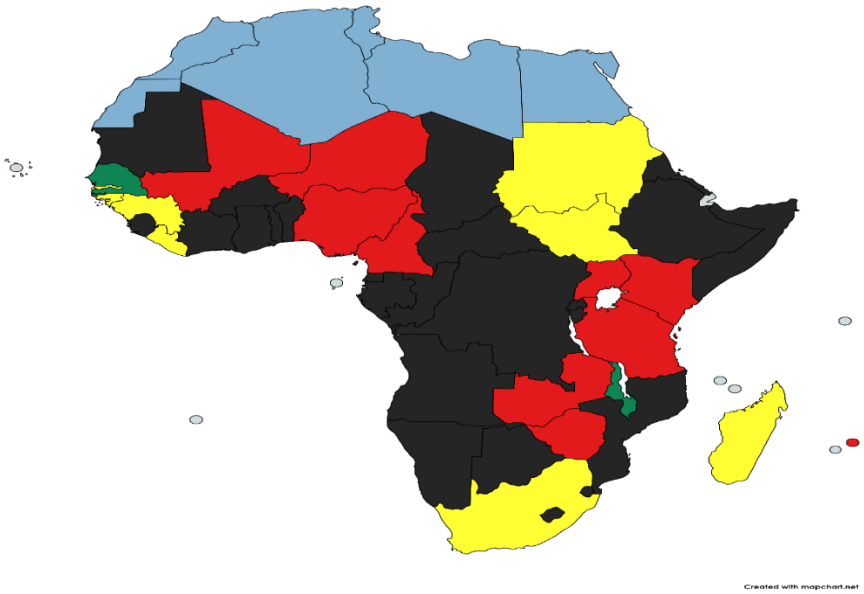

Figure 3: Showing the geographical location of the SSA countries where S. haematobium studies were conducted and diversity determined; Red represents countries reporting high genetic diversity of S. haematobium, green represents countries reporting moderate genetic diversity of S. haematobium, yellow represents countries reporting low genetic diversity of S. haematobium, black represents countries within SSA where no studies of genetic diversity of S. haematobium were reported and blue represents countries in North Africa not included in the study. (Note: Map made using map chart)

Table 1: Summary of articles selected in the current review showing the Molecular Epidemiology and Genetic Diversity of S. haematobium in Sub-Saharan Africa

Author/Year of Study	Country	Study Population/ Samples used	Method used to determine genetic diversity	Study objective	Key Findings
(26)	Zanzibar, Mauritius Nigeria, Malawi, South Africa, Senegal	Clonal cercariae from snails	Microsatellite markers	To develop numerous microsatellite loci from the *S. haematobium* genome for long term and short term use	1. Highest genetic diversity from Zanzibar and lowest diversity observed in samples from South Africa 2. Samples from Malawi, Mauritius and Nigeria were moderately diverse
(29)	Mauritius, Nigeria, Cameroon	Parasite adult worms	Microsatellite markers	To isolate and characterise *S. haematobium* DNA	High allelic diversity with alleles ranging from 2-7 and gene diversity from 0.29-0.76
(30)	Mali	Children/ Parasite egg	Microsatellite markers	To characterise population genetics of *S. haematobium* and identify the potential association of parasite and/ genotype with infection intensity	1. Limited evidence of population subdivision suggesting few barriers to gene flow in this population 2. Older children and boys harboured more diverse infections; unique adult genotypes present 3. Individual parasite genotypes had variable reproductive success across hosts
(15)	Mali, Nigeria	Primary school-aged children/ pooled egg samples	Microsatellite markers	To explore the differences in allelic diversity and composition among the populations in Mali and Nigeria	1. High levels of genetic variability demonstrated in Mali 2. Average allele number of individuals higher in Mali than Nigeria

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					3. No significant difference was seen in composition among the Nigerian Population 4. Allelic composition significantly different among Nigeria and Mali populations
(31)	Kenya Tanzania, Uganda, Niger, Mali, Cameroon	Children	Microsatellite markers	To use microsatellite markers to characterise the population genetic structure of *S. haematobium* from multiple locations across the African continent	1. High levels of genetic diversity documented 2. Allelic richness did not differ significantly among ten schools 3. High levels of heterozygote deficiency were seen in East Africa than in West Africa
\	Niger, Zanzibar	Children and Snail/ Miracidia from hatched eggs in urine samples and cercariae harvested from snails	Microsatellite markers	To develop novel multiplex microsatellite PCRs to enable high-throughput population genetic studies of *S. haematobium*	1. The number of alleles observed across the loci ranged from 2 to 33 2. High genetic diversity observed in the Pemba miracidial population compared to that from Niger 3. Cercariae obtained from each snail had identical genotypes showing that they were clonally derived from a single miracidium
(27)	Sudan	Children aged 6-17 years/ parasite eggs	RAPD	To identify if there is any relationship between genetic diversity of *S. haematobium* and pathology of disease in school children in Gezira state, Sudan	Three genotypes identified with no relationship between genetic diversity and pathology of the disease
(11)	Zimbabwe, South Africa	Parasite eggs from heavily infected patients	RAPD	To examine the possible diversity among *S. haematobium* using simultaneous amplification of genomic DNA of selected isolates	1. Moderate to high genetic diversity observed 2. *S. haematobium* split into two phylogenetic clusters 3. Genetic similarities observed among isolates from Zimbabwe and South Africa
(14)	Zimbabwe	Children aged 9-16 years	RAPD	To examine the relationship between genetic diversity and clinical outcome	1. 13 clusters of associated genotypes identified 2. Three clusters overrepresented in children with severe infection
(28)	Zimbabwe	children/ eggs hatched and cercariae harvested from snails	RAPD	To characterise the genetic diversity of *S. haematobium* in infected individuals	1. 53 unambiguous loci were produced from 4 primers, of which four were polymorphic 2. Diversity both within and between hosts 3. Parasite population segregated into 13 clusters of associated genotypes
(32)	Zimbabwe	Children and Snails/Laboratory passaged adult worms and cercariae	RAPD	To examine genetic diversity occurring in a population of schistosomes in Zimbabwe	1. A high degree of polymorphism shown 2. Analysis of molecular variance of 76.3 from five individuals suggests infection with multiple genotypes
(5)	Malawi	Children and Mothers	Mitochondrial DNA barcoding of cox1	To assess the risk and local epidemiology of *S. haematobium*	DNA barcoding revealed the presence of group 1 *S. haematobium*
(20)	Liberia, Gambia, Guinea Bissau, Coastal Kenya, Madagascar Zambia	Parasite eggs	DNA barcoding of Mitochondrial cox1 gene and nad1	To document the genetic variation of *S. haematobium* from different geographic locations in Africa	1. Low sequence variation in cox1 and nad1 2. 61 haplotypes were found within individual samples and split into two groups (groups 1 and 2) 3. The high occurrence of H1 haplotypes suggests that the population underwent genetic bottleneck followed by expansion
(33)	Zanzibar, Tanzania	Children/ Parasite Miracidia	DNA barcoding of cox1 and nad1	To establish the level of genetic variation of *S. haematobium* before and after control	1. Diversity was high with limited population structuring

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					2. Sequence variation detected in cox1 and nad1 with 27 and 22 haplotypes identified respectively 3. Haplotypes and barcodes types partitioned into two groups (group 1 and 2)
(34)	Nigeria	children/ Parasite eggs	DNA barcoding of Cox1	To establish the level of genetic diversity of *S. haematobium* among school children in Kebbi State, Nigeria	1. Prevalence was found to be 32.09% 2. Sequences were phylogenetically related to *S. haematobium* from Kenya and consistent with predominant species in Africa
(2)	Sudan	children	ITS2-RFLP	To characterise the genotype of *S. haematobium* in an infected population and identify the potential association with parasite diversity	Most of the *S. haematobium* in Sudan is of a pan- African origin with an inflow of Kenyan in White Nile, Sudan

Key: nad1: NADH-dehydrogenase subunit 1, Cox1: Mitochondrial Cytochrome Oxidase Subunit 1, RAPD: Randomly Amplified Polymorphic DNA, ITS2- RFLP: Internal Transcribed Spacer Region 2 Restriction fragment Length Polymorphism, PCR: Polymerase Chain Reaction.

During data analysis, SSA was divided into 4 regions, Central Africa, East Africa, West Africa and Southern Africa. From all the research papers on genetic diversity of *S. haematobium* that were added to the review, the majority were conducted in West Africa 15 (42%), followed by East Africa 13 (36%) and 8 (22%) from Southern Africa (Figure 4). There was no study conducted in Central Africa. Nigeria and Zimbabwe contributed the most studies 4 (11%) each), whereas Senegal, Madagascar, Liberia, Gambia, Guinea Bissau and Uganda contributed the least 1 (3%)) each (Table 2).

Table 2: Showing the number of studies conducted from the different countries of Sub-Saharan Africa

Study regions: East African countries- Zanzibar, Mauritius, Sudan, Madagascar, Kenya, Tanzania, Uganda, West African countries- Nigeria, Senegal, Cameroon, Mali, Liberia, Gambia, Guinea Bissau, Niger and Southern African countries- Malawi, South Africa, Zimbabwe

County	Frequency	Percentage (%)
Nigeria	4	11
Zimbabwe	4	11
Mali	3	8
Tanzania	3	8
Zanzibar	2	6
Mauritius	2	6
Malawi	2	6
South Africa	2	6
Sudan	2	6
Cameroon	2	6
Kenya	2	6
Niger	2	6
Senegal	1	3

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Madagascar	1	3
Liberia	1	3
Gambia	1	3
Guinea Bissau	1	3
Uganda	1	3

3. Discussion

The current review is aimed at determining the genetic diversity of *S. haematobium* in SSA countries in studies conducted throughout 2000-2020. Many studies were retrieved. However, only 16 met our inclusion criteria. Our findings in the current study indicate that the genetic diversity of *S. haematobium* in SSA countries is high. The current study has similarities with studies done elsewhere in Egypt [35]) and Yemen [36] where a high genetic diversity was also detected. Other studies have also shown that high genetic diversity is not only detected in *S. haematobium* but also in other parasites such as *S. mansoni* [37] and *S. japonicum* [16,38]**.** The high genetic diversity could be attributed to the continuous pressure imposed on the parasite by control measures such as MDA. It has been shown that MDA could lead either to low or high parasite diversity [12]. An increase in diversity indicates increased genetic exchange of a parasite population with other populations in different geographical areas and/or host species. In contrast, a reduction in diversity means that the parasites will be less likely able to adapt to environmental pressure, including chemotherapy. Other factors which are likely to affect the diversity of *S. haematobium* include overlapping contact sites, immunity and susceptibility to definitive and snail intermediate hosts [13,22]. Human movements can further promote the existence of variable genotypes in certain regions. This high genetic diversity can then lead to the emergence of parasites that are either non-susceptible or drug-resistant. This can happen through genetic swapping and recombination between old and newly emerged genotypes [22]. Nevertheless despite the observed high genetic diversity, the difference was not statistically significant indicating that the distribution in terms of genetic diversity is the same for the whole of sub- Saharan African countries.

Our results indicate that various methods have been used in different studies to determine the genetic diversity of *S. haematobium* and the most commonly used method is microsatellite markers followed by DNA barcoding of selected genes and RAPD. Only one study used ITS- RFLP. The reason for the increased use of microsatellites in the various studies could be because microsatellites are highly variable and specific and are also able to perform population-level analysis [21] and ideal for population genetic studies to identify clusters of genetically related individuals [39]. The microsatellite markers have been used in various studies for example in determining the genetic diversity of other parasites such as *S. mansoni* [31,40] and snails [41] and have yielded good results in comparison to other methods such as ITS-RFLP which has been shown not to give enough information on the genetic diversity of *S. haematobium* and hence may not be used as a genetic diversity marker for the parasite [20]. However, a statistical test to determine the association between genetic diversity determined by microsatellites only and SSA regions showed no statistical significance as most of the studies that used microsatellites for genetic diversity determination showed a high genetic diversity.

Our findings also indicate that the articles included in our study were published between 2000 and 2020 with the year 2012 having the highest number, 11 (30%) followed by the year 2013. This significant increase in these years could be attributed to the increase in the number of molecular tools used for genetic diversity determination which has enhanced molecular studies in these parasites [23]. The development of microsatellite mark-

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ers has also contributed to the enhancement of genetic diversity determination in *S. haematobium* and all parasites in general [13,21,29,30]. These and many other molecular tools have enhanced understanding of the population structure of *S. haematobium* and many other parasites which are continuously subjected to drug pressures [13,26]. The results of the current study are in agreement with findings on studies done in *S. mansoni* and *S. japonicum* which indicate that more studies have been done in these parasites compared to *S. haematobium*. For example, there had been a 10-fold difference in the number of papers published on *S. mansoni* in 40 years in comparison to *S. haematobium* and molecular studies on this parasite had lagged [23,30]. This had been due to the more demanding conditions for laboratory maintenance of *S. haematobium* and a lack of available molecular markers for the parasite [16,33].

In our study, after dividing SSA into four different regions, East, West, Central and southern Africa during data analysis, we found that more studies were conducted in West Africa followed by East Africa and the least number of studies were conducted in Southern Africa. No study was recorded in Central Africa. Nigeria in West Africa and Zimbabwe in Southern Africa contributed most of the studies while Senegal, Madagascar, Liberia, Gambia and Guinea Bissau contributed the least. The reason for the high number of studies in Nigeria and Zimbabwe could be because the disease is so prevalent in the two countries and there is active research being done while no active research is done in other countries despite the disease being very common [7,42]. Our findings are in agreement with other research which has shown that Nigeria has the highest prevalence of schistosomiasis followed by Tanzania in second place and then Ghana, the Democratic Republic of Congo and Mozambique making up the top five countries in SSA [42–44]. Despite the high prevalence of schistosomiasis in some of these countries, there is a paucity of studies that are done [42,43] and as shown in our current study no genetic diversity studies have been done in some countries where schistosomiasis is highly endemic. It has been noted that despite *S. haematobium* having a larger geographical distribution in Africa, parts of the Middle East, Madagascar and the Indian Ocean Islands, compared to other parasites, it has been a subject of very few genetic studies. This has been so even if genetic/region variation and suggested mixing of parasites strains due to human movements has been shown. It is therefore imperative that investigations are done to explain the extent of genetic variations in this parasite if its potential evolution and control is to be understood [14,20,30].

Systematic reviews have several benefits, but they are usually accompanied by challenges and limitations. For example, our search was limited to articles published in English. It is, therefore, possible that some publications relevant to this study may have been left out. The article also describes the genetic diversity of *S. haematobium* for countries in SSA only. Thus, it may not be of so much interest to researchers outside this region. However, this will serve as a reference for researchers within SSA who wish to research urinary schistosomiasis.

4. Materials and Methods

4.1. Selection Criteria

The available literature was systematically reviewed following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) protocol guidelines (S1 checklist) (24). The search strategy involved the identification of records through database searching followed by the screening of abstracts and assessment of records for eligibility as outlined in the PRISMA flow diagram (Figure 1).

The following keywords were used for the search; schistosomiasis, Schistosoma haematobium, urinary schistosomiasis, genetic diversity, population genetic structure and Sub-Saharan Africa (SSA). Boolean operators (AND, OR)

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were used when searching, and the search was limited to articles written in English. The records were identified through a search of databases, Google Scholar, African journals online and PubMed. Two independent authors screened the articles to select carefully literature that was eligible for the review.

4.2 Inclusion Criteria

Articles were included if they were published between 1st January 2000 and 31st July 2020 and conducted in countries within SSA. For studies that included data from non-SSA countries, only data from countries within SSA was extracted. Studies were included if they focused on genetic diversity, population structure and molecular characterization of S. haematobium in SSA in samples from humans and snail hosts.

4.3 Exclusion Criteria

Studies published before 1st January 2000 and after 31st July 2020, review articles, personal opinions, comments/letters to the editor, editorials, congress or conference abstracts and studies not written in the English language were not included. Studies were also excluded if they were published in countries outside Sub-Saharan Africa and if the method of diagnosis used in the study and source of the sample used was not specified. Studies that only focused on Schistosomiasis in general and did not focus on genetic diversity, molecular epidemiology and population genetics of S. haematobium were also excluded.

4.4 Data Extraction

The following information was extracted from studies that met the inclusion criteria: first author, year of publication, study population, study objectives, country of study and summary of findings.

4.5 Data Analysis

Data were entered in Microsoft Excel 2019 for Mac and analyzed using python 3.7 for mac. Descriptive data such as distribution of studies, method of genetic diversity determination were reported as frequencies and percentages and presented as tables and graphs. The fisher's exact was conducted to determine the relationship between SSA regions and the genetic diversity of *S. haematobium.*

5. Conclusion

Our study has shown that the genetic diversity of *S. haematobium* in SSA countries is generally high. However, Fisher's exact test showed that there is no significant difference in the distribution of genetic diversity in SSA regions. Different methods such as microsatellite markers, DNA barcoding and RAPD have been developed and microsatellite markers are the most commonly used method in determining the genetic diversity of *S. haematobium*. The current study has also shown that there has not been a significant increase in the number of studies conducted on genetic diversity for the period 2000-2020 with no study conducted in some parts of SSA despite recording a high prevalence of the disease. These results have therefore shown the need for conducting more research on the genetic diversity of *S. haematobium* as this will aid in providing information on the response of parasite populations to drug treatment pressures, get insight on the epidemiology of infection and advance novel treatment. This is especially important for SSA if eradication of the disease is to be achieved.

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