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

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ABSTRACT

Background: Urogenital 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 disease management. This review aimed to systematically review the scientific literature on the genetic diversity and population structuring of *S. haematobium* and the methods used across sub-Saharan African countries.

Methods: A literature search was done on PubMed, African Journals Online and Google scholar using predefined search terms such as urinary schistosomiasis, *S. haematobium*, and genetic diversity in sub-Saharan Africa in combination with Boolean operators (AND, OR). The search included studies published from 2000-2020 that emphasised on genetic diversity of *S. haematobium* in sub-Saharan Africa. A country in sub-Saharan Africa was included if it had a study that determined the genetic diversity of *S. haematobium*.

Results: Sixteen study articles from 18 sub-Saharan African countries met the inclusion criteria. The genetic diversity of *S. haematobium* varied from low to high using different methods. Most studies (18/36 or 50%) in these countries showed a high genetic diversity

of *S. haematobium*. Four methods, namely, restriction fragment length polymorphism, randomly amplified polymorphic DNA, DNA barcoding and Microsatellite markers, were used to determine diversity. In these studies, measures of genetic diversity such as number of alleles per minute or allelic richness, heterozygosity, number of genotypes and unique haplotypes were used. Microsatellites were the most commonly used method, and the studies reported a number of alleles per locus ranging from 2-19 alleles per locus and heterozygosity of 6 to 71% in some studies. The highest number of studies were conducted in West Africa, Nigeria and Zimbabwe, at 4/36(11%) each.

CONCLUSION

Results show the need for continued monitoring of genetic variations in *S. haematobium* in sub-Saharan Africa. This will aid in understanding disease epidemiology and advancing novel treatment and vaccine strategies.

Keywords: Urogenital schistosomiasis, *Schistosoma haematobium*, sub-Saharan Africa, Genetic Diversity, Systematic Review

BACKGROUND

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 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 constitutes 13% of the world's population (5,6). Schistosomiasis is caused by the trematode worms of the genus Schistosoma. The most common disease-causing organisms of this genus are *Schistosoma haematobium*, *S. mansoni* and *S. japonicum*. However, *S. haematobium* is the most widely distributed *schistosome* and is responsible for causing human u*rogenital schistosomiasis* (7,8).

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% of school-aged children at risk of morbidity by 2010 (1,4). 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 (9).

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 (10). The genetic diversity of parasites, defined as the variations or polymorphisms in the genetic composition of an organism, 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 (12). It may also be important in determining the disease's clinical outcome, ranging from mild symptoms to severe damage to the kidneys and/or bladder (13). The genetic diversity of parasites is an important factor that determines their potential to produce harmful effects among human or host populations they parasitise. 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 (13). Studying the genetic diversity of schistosome parasites allows for linking some genotypes with disease prevalence, and this can then be used to formulate effective control measures (14,15).

Praziquantel (PZQ) remains the drug of choice for schistosomiasis treatment and morbidity control (16,17). 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 (18). This is likely to place strong and novel selective pressures on the parasites, which may impact their population structure and genetic diversity (19).

In SSA, molecular methods such as mitochondrial DNA barcoding, microsatellite markers, restriction fragment length polymorphism and Randomly Amplified Polymorphic DNA (RAPD) are used for the determination of genetic diversity and population structure of schistosomes (12,19,20). 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 (21). 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* (19).

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 and gene flow 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 (20). Therefore, the current review aims to determine 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 the treatment, control and elimination of urinary schistosomiasis in SSA.

METHODS

Selection criteria

The available literature was systematically reviewed following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) protocol guidelines (S1 checklist) (23). The search strategy involved identifying records through database searching, followed by screening abstracts and assessing records for eligibility as outlined in the PRISMA flow diagram (Figure 1).

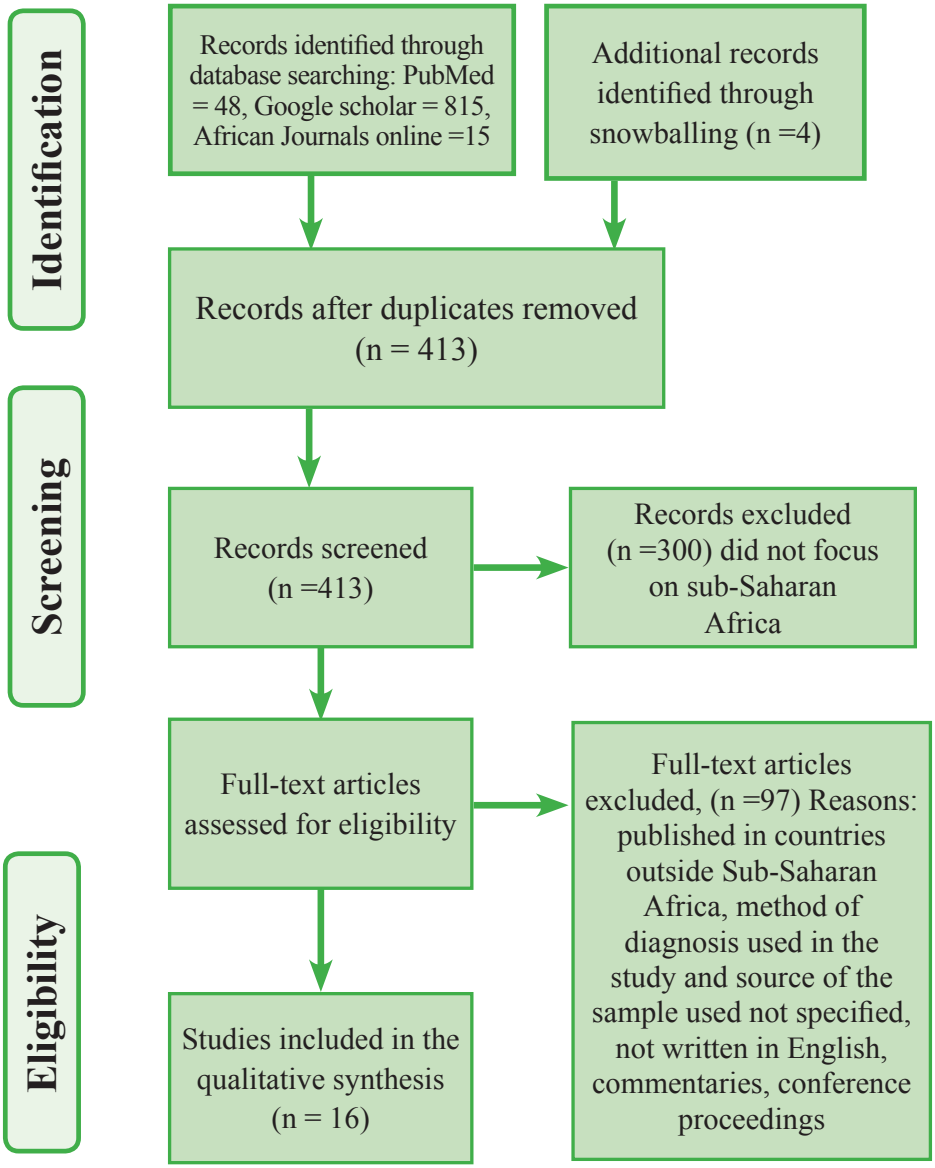

Figure 1: Preferred Reporting Systems for Systematic and Meta-Analyses (PRISMA) Flow Diagram for articles included in the current review

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) 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 literature that was eligible for review.

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 characterisation of *S. haematobium* in SSA in samples from humans and snail hosts. A country in sub-Saharan Africa was included if it had a study that determined the genetic diversity of S. haematobium.

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 English 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 were 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.

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 (Table 1).

Data Analysis

Data were entered in Microsoft Excel 2019 for Mac and analysed using python 3.7 for mac. Descriptive data such as distribution of studies and 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.

RESULTS

Search Results

Characteristics of studies included

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. The reviewed

articles were published between 2000 and 2017 in 18 SSA countries. The year 2012 has the most studies, 11(30%), followed by 2013, 8(22%). 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.

Table 1: Summary of articles selected in the current review showing the molecular epidemiology and genetic diversity of *S. haematobium* in sub-Saharan Africa

Key: nad1: NADH-dehydrogenase subunit 1, Cox1: Mitochondrial Cytochrome Oxidase Subunit 1, RAPD: Randomly Amplified Polymorphic DNA, ITS2-RFLP:

Country	*Study Population/ Samples used*	*Method used to determine genetic diversity*	*Study objective*	*Key Findings*	*Author/Year of study*
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. Genetic diversity is measured by the total number of alleles per locus, ranging from 5-15 alleles per locus. 2. Expected heterozygosity was 6% for South Africa and ranged between 51% and 70% for the other countries. 3. The highest genetic diversity from Zanzibar and lowest diversity were observed in samples from South Africa. 4. Samples from Malawi, Mauritius and Nigeria were moderately diverse	Glen *et al,.,* 2013 (27)
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	Golan *et al.,* 2014(28)
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. Highly polymorphic loci, the number of alleles per locus ranged from 8 to 19. 21 private alleles were detected in one child 2. Limited evidence of population subdivision suggests few barriers to gene flow in this population. 3. Older children and boys harboured more diverse infections; unique adult genotypes were present. 4. Individual parasite genotypes had variable reproductive success across hosts.	Gower *et al.,* 2011 (30)

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 were demonstrated in Mali 2. The average number of alleles per locus ranged from 2.3 to 5.9, and the highest recorded in Mali (5.8 alleles) at one locus 3. The average allele number of individuals is higher in Mali than in Nigeria 4. No significant difference was seen in composition among the Nigerian population 5. Allelic composition significantly different among Nigeria and Mali populations	Ezeh *et al.,* 2015 (14)
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 were documented. 2. High diversity was recorded in East African countries than in West African countries, a mean number of alleles per locus in each school ranged from 3.88 to 18.49. 3. There was significant differentiation between parasite populations different children within schools (Fst = 0.017, P < 0.001)	Gower *et al.,* 2013 (29)
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 2-33 2. High genetic diversity was observed in the Pemba miracidial population compared to that of Niger 3. Cercariae obtained from each snail had identical genotypes showing that they were clonally derived from a single miracidium	Webster *et al.,* 2015 (45)
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.	1. Genetic diversity is measured by the number of variable bands per sample. 2. Only three bands (three genotypes) were detected, indicating a small number of variant alleles. 3. No relationship between genetic diversity and pathology of the disease	Gasmelseed *et al.,* 2014 (34)

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. Diversity is measured by the banding patterns based on band sharing; bands lost in one isolate and present in the other. Moderate to high genetic diversity observed 2. More bands were shared by the isolates from Zimbabwe and South Africa (similarity index = 0.721) than those shared by each with the Egyptian isolate (similarity index = 0.551 and 0.566) 3. *S. haematobium* split into two phylogenetic clusters	Afifi *et al.,* 2016 (12)
Zimbabwe	Children aged 9-16 years	RAPD	To examine the relationship between genetic diversity and clinical outcome	1. 53 unambiguous loci, of which 22 (41.5%) were polymorphic 2. 13 clusters of associated genotypes were identified after segregation using modified cluster analysis 3. Three clusters are overrepresented in children with severe infection	Brouwer *et al.,* 2003 (13)
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 22 were polymorphic 2. Mean heterozygosity in the population was 0.116 ± 0.043. An analysis of molecular variance showed that most variances occurred within rather than between hosts. 3. Frequencies of certain alleles segregated the parasite population into 13 distinct clusters of associated genotypes, with	Brouwer *et al.,* 2001 (33)
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 is shown. 2. Seventy-one bands were amplified from five primers, and 37 (52.1%) were polymorphic. The mean level of heterozygosity was 0.212 from a population of 31 individuals from the same geographical area. 3. Analysis of molecular variance of 76.3 from five individuals suggests infection with multiple genotypes and variation occurring within hosts.	Shiff *et al.,* 2000 (39)

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 only group 1 *S. haematobium* with the absence of group 2	Poole *et al.,* 2014 (5)
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 1978 individual samples and split into two groups (groups 1 and 2) 3. The high occurrence of H1 haplotypes suggests that the population underwent a genetic bottleneck followed by expansion	Webster *et al.,* 2012 (19)
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 2. Sequence variation was detected in cox1 and nad1, with 27 and 22 haplotypes identified, respectively 3. Haplotypes and barcodes types partitioned into two groups (groups 1 and 2) using phylogenetic analysis	Webster *et al.,* 2012 (25)
Nigeria	children/ Parasite eggs	DNA barcoding of Cox1	To establish the level of the genetics 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.	Umar *et al.,* 2017 (26)

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. The majority of the studies were conducted in West Africa 15/36 (42%), followed by East Africa 13/36 (36%) and 8/36 (22%) from Southern Africa. There was no study conducted in Central Africa. Nigeria and Zimbabwe contributed the most studies 4/36 (11%) each), whereas Senegal, Madagascar, Liberia, Gambia, Guinea Bissau and Uganda contributed the least 1/36 (3%)) each (Table 2).

Table 2: Studies conducted in different countries in sub-Saharan Africa

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

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

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

Genetic Diversity of *S. haematobium*

The majority of the countries showed high genetic diversity of *S. haematobium* with 18/36 (50%) studies showing a high genetic diversity (high number of genetic variations or polymorphisms) followed by low 10/36 (28%) and moderate 4/36 (11%) in various regions of SSA.

However, the 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. No statistically significant association between region and genetic diversity was found (p=0.84).

Methods of genetic diversity

The current study results show that various methods were used to determine the genetic diversity of *S. haematobium;* the most commonly used method was microsatellite markers, followed by RAPD. The following was the distribution of the methods used: microsatellites were used in 6/16 studies (37.5 %) studies, RAPD in 5/16 (31.3%) studies, DNA barcoding of mitochondrial genes in 4/16 (25%) studies, nuclear ITS2- RFLP in 1/16 (6.3%) study. The major findings of the 16 study articles in the current review and the measures of genetic diversity employed in the studies are summarised in Table 1.

DISCUSSION

This review aims to provide an overview of the genetic diversity of *S. haematobium* in SSA countries and the methods used to determine genetic diversity in studies conducted from 2000-2020. Sixteen articles met the inclusion criteria. The studies included in this review indicated that the genetic diversity of *S. haematobium* varied across and within countries from low to high, and the diversity of the parasite varied depending on the method used and not on the type of samples used. However, most of the studies reviewed indicated the high genetic diversity of the parasite. The results obtained in this review corroborate findings from Egypt, a country outside SSA in which high genetic diversity was detected using RAPD.

Moreover, high genetic diversity has been detected in other schistosomes parasite species such as *S. mansoni* and *S. japonicum* (15,25,35). 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 can lead either to low or high parasite diversity (10). An increase in diversity indicates increased genetic exchange of a parasite population with other populations in different geographical areas 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(10). Other factors which are likely to affect the diversity of *S. haematobium* include overlapping contact sites, parasite inbreeding and interbreeding, genetic bottleneck, founder effect, immunity and susceptibility to definitive and snail intermediate hosts (11,19,21). 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 (21).

The results of the current review indicate that four methods were used to determine the genetic diversity of

S. haematobium in SSA countries. The most commonly used were microsatellite markers. High genetic diversity or sequence variation was indicated in all the studies that used this method. Microsatellites or short tandem repeats (STRs) are repetitive DNA motifs ranging in length from two to six base pairs that are tandemly repeated 5-50 times and occur at thousands of locations within the genome. They have a higher mutation rate than most other genome regions, leading to high genetic diversity(36). Microsatellite markers have also been recommended for use in population genetics and genetic diversity studies of schistosome parasites because they are co-dominantly expressed and serve as neutral markers. They can also show heterozygosity and a reasonable number of alleles per polymorphic locus in samples, making them a better method than RAPD (15,31). They also provide opportunities to investigate important topics such as relationships between parasite genetics and disease burden and the consequences of various control activities on parasite populations (10).

The findings of this review indicate that RAPD-PCR technology was the second most commonly used method for determining the genetic diversity of *S. haematobium*. Using this method, results ranging from low to high genetic diversity were obtained. RAPD primers have allowed it to conduct population-based studies of schistosomes with very little prior sequence information. These primers have also been widely used to examine the genetic diversity among populations of schistosomes in snails (34,39,40). These primers can screen through a large number of loci within the entire genome of an organism and have proven to help characterise inter and intraspecific relationships. The RAPD-PCR requires minimal DNA material; hence, cercarial or miracidial stages can be used, minimising the selection bias caused by laboratory passaging of parasites through unnatural hosts(34). However, the major drawback of this method is in the segregation of dominant markers in which heterozygous and homozygous dominant individuals show the same banding patterns and homozygous recessive individuals show no banding patterns at a locus (41).

DNA barcoding using mitochondrial genes cox1 and nad1 was the third most commonly used method in determining the diversity of *S. haematobium*. Studies that used this method indicated low sequence variation or genetic diversity, hence showing that *S. haematobium* is less diverse compared to its sister species, S. *mansoni*. Using DNA barcoding, *S. haematobium* has been split into two phylogenetic groups compared to *S. mansoni*, which has been slit into five lineages (19,37). The low sequence variation in DNA barcoding of mitochondrial genes owes to the fact that genetic content and gene order in mitochondrial genes are conserved among species. Sequences from mitochondrial genomes are, therefore, usually used for defining population groups, tracing the genetic history of an individual or a particular group of related individuals, and constructing deep-branch taxonomic phylogenies (38).

The current review further indicates that RFLP of the nuclear ITS gene was used in only one study conducted in Sudan using *S. haematobium* egg samples. No

variation in size was seen among the fragments detected after the ITS gene was subjected to RFLP-PCR analysis. This shows that the *S. haematobium*. ITS gene is usually monomorphic, shows no sequence variation across all samples, and may not give enough information in genetic studies (19). To solve this problem, RFLP-PCR analysis has been used to detect polymorphisms in the ITS gene and also used to distinguish *S. haematobium* from its sister species, S. bovis. RFLP-PCR uses enzymes to identify variations or single nucleotide polymorphisms in homologous DNA. The DNA is isolated and fragmented into different lengths using enzymes. These differences in lengths of fragments of DNA between restriction sites are used to quantify genetic diversity in populations. The polymorphisms in homologous DNA cause differences in fragment length and are used to distinguish individuals, populations, or species(42). However, this method requires large amounts of DNA; hence, DNA may not be sufficient, especially when using cercarial or miracidial DNA (2).

Our findings also indicate that the articles included in our study were published between 2000 and 2020, with 2012 having the highest number, 11 (30%), followed by 2013. This significant increase in these years could be attributed to the increase in the number of molecular tools used to determine genetic diversity (22). The development of microsatellite markers has also contributed to the enhancement of genetic diversity determination in *S. haematobium* and all parasites in general (11,20,28,30). These and many other molecular tools have enhanced our understanding of the population structure of *S. haematobium* and many other parasites which are continuously subjected to drug pressures (11,27). 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 on 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 compared to S. haematobium, and molecular studies on this parasite had lagged behind (22,30). This has been due to the more demanding conditions for laboratory maintenance of *S. haematobium* and a lack of available molecular markers for the parasite (15,25).

The current review also indicates that the highest number of studies were conducted in Nigeria and Zimbabwe. The reason for the high number of studies in Nigeria and Zimbabwe could be due to the fact that 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(9,43). Our findings agree with prevalence studies in Nigeria, which have shown that Nigeria has the highest prevalence of schistosomiasis, followed by Tanzania in the second place and then Ghana, the Democratic Republic of Congo and Mozambique making up the top five countries in SSA (9,43,44). Despite the high prevalence of schistosomiasis in some of these countries, there is a paucity of studies that are done(9,43), 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 have been confirmed. It is, therefore, imperative that investigations are done to explain the extent of genetic variations in this parasite if its potential evolution and control are to be understood (13,19,30).

Systematic reviews have several benefits, but challenges and limitations usually accompany them. 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 urogenital schistosomiasis.

CONCLUSION AND RECOMMENDATIONS

This review has shown that the genetic diversity of *S. haematobium* varies from low to high within and across SSA countries, depending on the method used. Four methods, namely microsatellite markers, DNA barcoding of mitochondrial genes, RAPD and ITS gene RFLP- PCR. Microsatellite markers have been recommended for use in genetic diversity and population studies as they are a powerful and highly variable marker compared to the other method. 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 into the epidemiology of infection and advance novel treatment. This is especially important for SSA if eradication of the disease is to be achieved.

Conflict of Interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Conceptualisation, RT and AMP; methodology, RT; software, PN; validation, RT, MK and PN; formal analysis, P, N and RT; investigation, RT; data curation, PN; writing—original draft preparation, RT; writing—review and editing, PN, MZ, FM, MK, JY, CSS, HH, KSN, AMP; visualisation, RT, MZ, FM and PN, MK; supervision, AMP, JY, CSS, HH and KSN; funding acquisition, RT All authors have read and agreed to the published version of the manuscript.

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