Chromatographic and computational studies of ligands associated with bilharziasis

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Abstract

Objective: Rapid diagnostic techniques that do not depend on microscopic analysis are urgently needed for rapid diagnosis and management of bilharziasis. Specific ligands that are excreted through urine in bilharziasis may serve as rapid diagnostic biomarkers to replace microscopy, which is cumbersome and time-consuming. The aim of this study was to identify ligands associated with bilharziasis.

Methods: Microscopy was employed to detect ova of Schistosoma haematobium in urine specimens obtained from 1032 subjects. Pooled positive urine samples and pooled normal urine samples were separately prepared in triplicates and analyzed by gas chromatography-mass spectrophotometry (GC-MS). Ligands identified in each pool were noted. Computational analysis was performed between the schistosome receptors and ligands.

Results: GC-MS revealed that the level of indole in bilharziasis sample was higher than that in normal urine. Indole was the ligand with the highest (28.63%) concentration in the pooled positive urine sample, while ethyl phenazone level was the highest (69.64%) in the pooled normal sample. Computational analysis depicted perfect docking with indole and all other ligands identified in positive urine samples.

Conclusion: This study identified some ligands associated with bilharziasis some unique to normal (negative) urine samples.

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Introduction

Bilharziasis, otherwise known as urinary schistosomiasis, is caused by a digenetic trematode called *Schistosoma haematobium*. It is a tropical disease ranking second among the most widely distributed neglected tropical diseases.\(^1,2\) Previous studies\(^3,5\) have reported that bilharziasis is a public health concern worldwide and especially, in Sub-Saharan Africa. In sub-Saharan Africa, bilharziasis is second to plasmodium infections in terms of death rate and morbidity.\(^6-10\) Remote sensing and geographic information systems (GIS) have been employed to assess the prevalence of bilharziasis based on reports from past studies.\(^14,15\) According to the report of the World Health Organization, 200–300 million people in 74 countries have been affected with the disease and a further 500–600 million are exposed to infection risk.\(^6,12,16-18\) In Nigeria, infection is very common, with an estimated 11 million infected people, while about 101 million people are at risk for infection.\(^5\)

Hassan and his colleagues reported that urinary and intestinal schistosomiasis were probably more common in northern Nigeria, but subsequent reports showed that the distribution cuts across the entire country.\(^19\) *S. haematobium* is common in the Middle East and some parts of Europe, especially among travellers and migrants.\(^20\) The World Health Organization earlier reported that bilharziasis ranked second to malaria in terms of socio-economic and public health importance, particularly in tropical and sub-tropical countries. The main signs and symptoms of the disease include dysuria, haematuria, granulomatous host response, and urinary egg excretion.\(^21\) Parasitic DNA has been detected with polymerase chain reaction (PCR)-based methods and the method has been shown to be sensitive and specific. This technique is, however, very expensive and time-consuming.\(^22,23\) Microscopic detection of ova in urine is a routine method for bilharziasis diagnosis in high-prevalence settings.\(^18,24\); however, this method is tedious and requires expertise. Therefore, rapid diagnostic techniques that do not depend microscopic analysis are needed for rapid diagnosis and effective management of the infection, especially in endemic regions.

It is expected that some specific ligands excreted through urine during schistosomiasis may serve as rapid diagnostic biomarkers for bilharziasis to replace microscopy, which is labor-intensive and time-consuming. Diagnostic kits based on biomarkers can be manufactured in the form of test strips and cassettes. The aim of this study was to identify ligands associated with bilharziasis.

Materials and Methods

Sample collection

Clean, dry, leak-proof, and wide-mouthed plastic specimen containers were given to each participant, along with instructions on how to collect terminal urine samples without contaminants. Pre-designed questionnaires on personal data and morbidities were administered to the children. Approximately 15 ml of urine was collected in the containers from participants between 10:00 am and 2:00 pm on each collection day. The specimens were appropriately labelled with identification numbers and arranged in a cold box for conveyance to a departmental multipurpose laboratory for analysis. Overall, 1032 urine samples were collected from 654 to 378 apparently healthy male and female children, respectively, aged 5–13 years, from Olorunda and Irepodun, communities near Osogbo, Nigeria.

Microscopy and chromatographic analysis of urine samples

The samples of urine were centrifuged at 3000 revolutions per minute (rpm) for 5 min, and a drop of the re-suspended deposit was placed on a clean, grease-free slide and examined under a light binocular microscope using the \( \times 10 \) and \( \times 40 \) objectives, according to the method described by Chess-bough.\(^25\) For chromatographic analysis, three drops of 25% sulpho-salicylic acid were mixed with 5 ml of each urine sample and centrifuged gently at 3000 rpm for 5 min. Protein precipitates from urine samples positive for schistosome ova were pooled in clean universal bottles in triplicate.

Each pool was prepared from at least 40 positive urine samples. The same procedure was repeated for normal urine samples.

Protein analysis using gas chromatography-mass spectrophotometry (GC-MS): (a) Each protein pool was suspended in 5 ml of distilled water and injected into a mass spectrometer using a 5-ml syringe. The machine was switched on for an hour to allow for processing. (b) After processing, the retention time and percentage area of each ligand in the injected mixture displayed on the screen was printed and recorded.

In silico analysis

a) Downloading the receptor and ligand files

The receptor file was downloaded from [www.rcsb.pdb.org](http://www.rcsb.pdb.org) with the file extension.pdb (i.e., *S. haematobium* was downloaded with the identity number 2F8F.pdb). The crystal structure of 2F8F was deposited in pdb.org. The ligand files were downloaded from [pubchem.ncbi.nlm.nih.gov](http://pubchem.ncbi.nlm.nih.gov) and subsequently converted to pdbqt using Open Babel.

b) Conversion of ligand files for in-silico molecular docking analysis
Ligand files were converted from inchi to pdbqt format using Open Babel. In the Input format menu of Open Babel, the ligands with the file extension .inchi were opened to convert them to pdbqt format. From the Convert menu, the following three options were selected: Generate 2D coordinates, Generate 3D coordinates, and Preserve atom indices from input file.

The Convert button was clicked, and the output file was generated in pdbqt format. AutoDock Vina was used for the in silico analysis. A configuration file was prepared which encompassed the input file, the receptor, and the ligand files. The receptor and ligand files were prepared with the extension of atomic coordinate, partial charge, and solvation parameters (pdbqt). AutoGrid was used to search and locate the binding site/pocket where the ligand binds to the receptor. The pocket was recognized as a three-dimensional structure with x, y, and z dimensions and coordinates.

c) Preparation of receptor and ligand files for in silico analysis following the installation of AutoDock Vina and MGLTools (AutoDock and PyMol in the computer) the following crucial steps were followed one after the other in preparing the receptor files before conducting simulations of docking analysis:

i) The AutoDock tool was double-clicked to open it.

ii) The File menu of the AutoDock tool was opened, and Read Molecule was chosen from the drop-down menu, then on My Document (or where the receptor.pdb was saved after downloading it from the protein database). Most receptor structures from the protein database do not have hydrogen, so polar hydrogen was added. During this process, the receptor molecule was already displayed on the AutoDock main screen.

iii) From the drop-down menu in the Edit menu, Hydrogen was clicked, followed by Add Hydrogen and then Polar Only (then on polar hydrogen was already present in the receptor). To set the binding pocket of the receptor, AutoGrid was used.

iv) Grid was clicked, followed by Macromolecules, and then Close. In the popup box that appeared, a protein of interest was highlighted (i.e., the receptor molecule) and Select Molecule was clicked. From the dialogue box that appeared, OK was clicked. The receptor file was saved with extension pdbqt in the same folder where it was selected.

v) Grid was clicked again, followed by Grid Box. This step was performed to locate and choose the part of the receptor where the ligand bound. It involved choosing the dimensions and size of the binding pocket of the receptor, which are the centre (x), centre (y) and centre (z). Furthermore, the sizes of the binding pocket were represented as size (x), size (y), and size (z).

vi) Next, Ligand was clicked, following by Input, and Open. At this point, the ligand appeared on the main screen (to be able to view the ligand molecule well, the receptor molecule was hidden). There were rotatable and non-rotatable bonds in the ligand structure. The rotatable bonds were converted to non-rotatable bonds to generate a rigid receptor. This was achieved by clicking Torsion Tree, followed by Choose Torsion. The torsion number was displayed on the bonds. Clicking any of the numbers changed it to rotatable or non-rotatable. Clicking on Done finalized the structure.

vii) To save the ligand, Ligand was clicked (top left), followed by Output, and then Save as pdbqt. The file was saved in the same folder where it was selected. Finally, the docking simulation analysis was ready.

Results

Of the 1032 samples investigated, 390 (37.8%) were positive for bilharziasis, with 135 (13.1%) from Oke-Ore, 171 (16.5%) from Ilie, and 84 (8.1%) from Eko-Ende communities. Table 1 depicts the intensity of the parasite load in the three communities. In total, 210 (20.3%) school children had light infections, while 180 (17.4%) had heavy ones. Statistical analysis by ANOVA revealed a significant difference in the intensity of the infection according to the community (P = 0.0001, p-value < 0.05).

The frequency of *S. haematobium* based on the appearance of haematuria and schistosome ova in urine is presented in Table 2. Of the 1032 pupils examined, 315 (30.5%) tested positive for haematuria, while 390 (37.8%) were positive for schistosome ova. All pupils who tested positive for haematuria were also positive for *S. haematobium*. Ilie had the highest prevalence of infection (51.0% haematuria and 58.2% *S. haematobium* infection), followed by Eko-Ende (23.0% haematuria and 37.8% schistosome ova), and Oke-Ore (22.1% haematuria and 26.2% schistosome ova). The data revealed a slightly higher infection among female (38.1%) than male children (37.6%), but the difference was not statistically significant (P = 0.057; p-value > 0.05).

Distribution of ligands in the pooled positive urine specimens is shown in Table 3, indicating the percentage area covered and retention times of all the ligands identified in the GC-MS analysis. Indole showed the highest percentage area of 28.63%, implying that it is the most frequently occurring ligand in the pooled positive urine samples of the six ligands identified. The other prominent ligands were 3-hydroxybenzoic acid, heptasiloxane, tetrahydroxybenzoic acid, benzaldehyde, and silane.

Table 4 presents the distribution and retention times of all the ligands identified in the GC-MS examination of pooled normal urine samples. Indole presented the lowest retention time of 5.274 min, which was the time it took the

### Table 1: Intensity of bilharziasis with respect to communities investigated.

| Communities | Number examined | Positive | Light infection (%) | Heavy infection (%) |
|-------------|----------------|----------|---------------------|--------------------|
| Oke-Ore     | 516            | 135      | 90 (66.7)           | 45 (33.3)          |
| Ilie        | 294            | 171      | 66 (38.6)           | 105 (61.4)         |
| Eko-Ende    | 222            | 84       | 54 (64.3)           | 30 (35.7)          |
| Total       | 1032           | 390      | 210 (53.8)          | 180 (46.2)         |

Light infection: 1−49 eggs/10 HPF of urine (P < 0.05).

Heavy infection: >50 eggs/10 HPF of urine.

HPF: High Power Field.
GC-MS machine to identify a particular ligand, while Ethyl-phenazone had the highest percentage area of 69.64%, indicating that it was as the most frequently occurring. Other ligands identified in the pooled negative urine were isonicotinyl, phenol, quinoline, butanone, pyrazole, and L-citrulline.

**In silico analysis**

After docking simulations of each of the ligands identified by the GC-MS analysis, the predicted structures with the lowest binding energies for the pooled positive urine sample is shown in Figure 1. The lower the binding energy, the higher the stability between ligand and receptors. Indole showed the lowest binding energy of $-4.0$ kcal/mol, and thus created a more stable bond with the schistosome receptor. Similarly, the predicted structures of ligands identified in the GC-MS analysis of pooled normal (negative) urine samples are depicted in Figure 2. Indole and ethylphenazone had the highest concentrations in the pooled positive (Figure 1) pooled normal (Figure 2) urine samples, respectively.

**Table 2: Association between haematuria and bilharziasis in relation to gender.**

| Sex     | Number examined | Number with haematuria (%) | Number with Schistosoma ova (%) | Number without Schistosoma ova/haematuria (%) |
|---------|-----------------|----------------------------|---------------------------------|-----------------------------------------------|
| Male    | 654             | 201 (30.7)                 | 246 (37.6)                      | 408 (62.4)                                    |
| Female  | 378             | 114 (30.2)                 | 144 (38.1)                      | 234 (61.9)                                    |
| Total   | 1032            | 315 (30.5)                 | 390 (37.8)                      | 642 (62.2)                                    |

**Table 3: Ligands in pooled positive urine samples.**

| Ligands               | Retention time (minutes) | Area (%)  |
|-----------------------|--------------------------|-----------|
| Indole                | 5.281                    | 28.63     |
| 3-Hydroxybenzoic acid | 5.481                    | 5.481     |
| Heptasiloxane         | 5.606                    | 23.77     |
| Tetrahydroxybenzoic acid | 6.025                  | 9.38      |
| Benzaldehyde          | 6.163                    | 10.63     |
| Silane                | 6.294                    | 11.70     |

**Table 4: Ligands in pooled normal urine samples.**

| Ligands               | Retention time (minutes) | Area (%)  |
|-----------------------|--------------------------|-----------|
| Indole                | 5.274                    | 9.81      |
| Isonicotinyl          | 5.694                    | 2.62      |
| Phenol                | 5.731                    | 3.77      |
| Quinoline             | 6.050                    | 4.85      |
| Butanone              | 6.156                    | 3.18      |
| Pyrazole              | 6.632                    | 3.26      |
| L-citrulline          | 7.445                    | 2.86      |
| Ethylphenazone        | 8.290                    | 69.64     |

**Discussion**

Overall, the rate (37.8%) of bilharziasis recorded in the present study is concerning and highlights the need for adequate management. This rate is, however, lower than that reported previously in pupils in Ilewo-Orile (58.1%), a rural community very close to Abeokuta in Ogun state, Nigeria, and in the Ezza North community (79.4%) in Ebonyi State, Nigeria. These differences could be attributed to the types of water bodies; abundance of snail vectors; salinity of the water; differences in ethno-geographical zone; cultural background; usage of rivers, streams, and ponds for domestic activities; and water contact practices of pupils.
The present study also found a slightly higher infection rate among female (38.1%) than male (37.6%) pupils. The difference was, however, statistically insignificant ($p = 0.057$, $p$-value $> 0.05$), implying that the distribution of bilharziasis is independent of gender. This observation could possibly be because children of the same age group, regardless of gender, generally involve themselves in similar activities involving water contact.

GC-MS was performed on the pooled positive and negative samples to assess the distribution of ligands. Indole recorded the highest percentage area of 28.63%, implying that it was the most abundant of the six ligands identified in the pooled positive urine samples. The other ligands were 3-hydroxybenzoic acid, heptasiloxane, tetrahydroxybenzoic acid, benzaldehyde, and silane. In pooled normal (negative) urine, analysis revealed that Indole had the lowest retention time of 5.274 min, which was the time it took for the GC-MS machine to identify the ligand, while ethylphenazone presented the highest percentage area of 69.64%, implying that it was the most abundant among the eight ligands identified in the pooled negative samples.

Other ligands in pooled negative urine were isonicotinyl, phenol, quinoline, butanone, pyrazole, and L-citrulline. The differences in the ligands identified could be attributed to the fact that the presence of schistosome may have suppressed the expression of some ligands and upregulated others. For instance, indole, 3-hydroxybenzoic acid, heptasiloxane, tetrahydroxybenzoic acid, benzaldehyde, and silane were abundant in pooled positive samples but suppressed in pooled negative samples; in contrast, indole, isonicotinyl, phenol, quinoline, butanone, pyrazole, l-citrulline, and ethylphenazone were abundant in pooled negative samples but suppressed in positive samples. This difference in the ligand levels could serve as marker for the diagnosis of bilharziasis.

The in-silico study showed perfect docking between ligands and schistosomes, thus corroborating the findings of the GC-MS analysis. The 3D structures obtained differ from one another because the structure of the receptor ($S. haematobium$) is constant, while the ligands are different because the ligands often adjusted to the shape of the receptor to allow successful docking with the lowest binding energy. In the absence of docking, none of the docking tools will run, there will be no binding between the ligand and receptor, and both molecules will appear separately.

Judging from the 3D structures generated for ligands identified in the pooled positive and negative urine samples, indole detected in the positive samples had the lowest binding energy of $-4.2$ kcal/mol, indicating a stable bond with the schistosome receptor, although indole was identified in both pooled positive and pooled negative samples. The binding energy in the pooled positive sample was lower than that in the pooled negative sample ($-7.8$ kcal/mol).

The 3D structures obtained from the in-silico study varied for each ligand because the receptor of $S. haematobium$ was constant, while the shape of the ligands changed. In addition, the shape of a receptor shape is usually adapted to promote successful docking with the appropriate binding energy. It is therefore clear from this study that indole is more strongly associated with pooled positive samples than with the pooled negative samples, as depicted by the lower binding energy.

The in silico studies carried out in this work indicated that all the bioactive components of the samples formed a good protein-ligand complex. This is an indication that they are good modulators of the protein. The position of the ligands in the receptor binding sites, as shown in Figures 1 and 2, show that they formed a complex with the ligands.

Our findings revealed that the presence of blood in urine had an insignificant effect on the types of ligands associated with normal urine or urine that contained $S. haematobium$ ova. Similarly, the abundance of $S. haematobium$ ova did not affect the types of ligands identified in urine samples.

This study may provide useful information for the development of rapid diagnostic strips and cassettes based on ligand biomarkers for bilharziasis, thereby eliminating the dependence on microscopic techniques for diagnosis, especially in rural areas, according to the report of Akinwale and his associates. However, the ligands present in the pooled positive sample could be due to acquired injury caused by laceration of the bladder walls: proteolytic enzymes produced by schistosome ova during laceration result in inflammation, which damages the urinary bladder.
In conclusion, the prevalence of bilharziasis among active school children was 37.8%. Some ligands such as indole, 3-hydroxybenzoic acid, heptasiloxane, tetrahydroxybenzoic acid, benzaldehyde, and silane were identified in normal with bilharziasis, while isonicotinyl, phenol, quinoline, butanone, pyrazole, and l-citrulline were identified in normal (negative) urine. Of note, the ligands identified in this study may only be applicable for detection of bilharziasis and not necessarily for diagnosis of intestinal schistosomiasis since the scope of the study was limited to analysis of urine samples. Therefore, similar studies using stool samples should be conducted to identify biomarkers for intestinal schistosomiasis. The ligands identified in this study were characteristic and specimen-dependent; while some were found only in positive urine samples, others were identified in normal specimens and few were identified in both positive and normal samples. Different ligand markers can be incorporated into a ligand-based diagnostic device to detect either positive or normal samples, depending on the band where the reaction occurs on the strip. In some cases of low-intensity infections that may not be detectable by microscopy or in rural areas with limited laboratory facilities, a rapid diagnostic technique may be highly advantageous. The cost of such a urinary schistosomiasis rapid test is expected to be moderate, allowing it to compete favourably with the currently used microscopy-based tests.

Lastly, the study recommends further research on proteomic identification of proteins associated with bilharziasis and confirmation of the present findings through larger multi-centre studies since the concept of a rapid diagnostic test for detection of schistosomiasis is still relatively new.

Source of funding

The study was self-sponsored and has not attracted grants from any funding organizations in the private or public sectors.

Conflict of interest

We affirm that there is no conflict of interest of any kind among all authors and that the manuscript has not been presented in any form or sent to any journal for publication.

Ethical approval

Prior to commencement of sample collection, ethical approval was sought and obtained from the Ethical and Animal Care Committee of the School of Basic Medical Sciences, Kwara State University (Reference Number KW/SBMS/ERC/018). The head of each community was visited and pre-informed about the study. Following the permission and consent of the heads, community residents, parents, and school-teachers, in accordance with the Helsinki declaration, only subjects in primary schools in the selected communities that volunteered were considered in the study.

Authors’ contributions

AAA conceptualized and designed the research. TDA and ION reviewed the research proposal and conducted the research, together with AB and KUE, while AB and TDA collated, organized, and analysed the data. AAA supervised the research methodology and data analysis. The initial draft of the article was written by the joint efforts of AB, KUE, and ION, while the final write-up was prepared by AAA. The final manuscript was properly reviewed and approved by all authors. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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References

1. Hotez PJ, Kamath A. Neglected tropical diseases in Sub-Saharan Africa: review of their prevalence, distribution and disease burden. J Negl Trop Dis 2009; 3(8): 412.
2. Rupp J. Female genital schistosomiasis from case report to a call for concerted action against this neglected gynaecological disease. Int J Parasitol 2016; 43(7): 395–404.
3. World Health Organization. The control of schistosomiasis. Report of the WHO expert committee. Geneva: WHO Technical Report Series; 2015.
4. Monde C, Syampungani S, van den Brink PJ. Natural and human induced factors influencing the abundance of Schistosoma host snails in Zambia. Environ Monit Assess 2016; 188(6): 1–14.
5. World Health Organization. Global health estimate : disease burden by cause, age, sex, country and by region 2000-2016, Geneva: Report of the WHO Expert Committee; 2017.
6. World Health Organization. Methods and data sources for global burden of disease estimates. Geneva: WHO Technical Report Series, WHO/HIS/HIS/GHE/2016; 2018.
7. King CH. Long-term outcomes of school-based treatment for control of urinary schistosomiasis: a review of experience in Coast Province, Kenya. Memórias do Inst Oswaldo Cruz 2006; 101(1): 299–306.
8. Koukounari A, Gabrielli AF, Touré S, Bosqué-Oliva E, Zhang Y, Sellin B, Donnelly CA, Fenwick A, Webster JP. Schistosoma haematobium infection and morbidity before and after large scale administration of praziquantel in Burkina Faso. J Infect Dis 2007; 196: 659–669.
9. Koukounari A, Webster JP, Donnelly CA, Bray BC, Naples J, Bosompem K, Shiff C. Sensitivities and specificities of diagnostic tests and infection: prevalence of Schistosoma haematobium estimated from data on adults in vilages northwest of Accra, Ghana. Am J Trop Med Hyg 2009; 80(3): 435–441.
10. Rudge JW, Stootherd JR, Basañez MG, Mgeni AF, Khamis IS, Khamis AN, Rollinson D. Micro-epidemiology of urinary schistosomiasis in Zanzibar: local risk factors associated with distribution of infections among schoolchildren and relevance for control. Acta Trop 2008; 105: 45–54.
11. King CH, Bertsch D. Meta-analysis of urine heme dipstick diagnosis of Schistosoma haematobium infection. Including low prevalence and previously-treated populations. PLoS Negl Trop Dis 2013; 7(9): 2431.
12. World Health Organization. The control of schistosomiasis. Second report of the WHO expert committee. Geneva: WHO Technical Report Series; 2013.
13. Chester K, Moses JC, Samson M. Schistosomiasis in Zambia: a systematic review of past and present experiences. *Dis. Poverty* 2018; 7:41.

14. Midzi N, Sangwene D, Zinyowera S, Mapingure MP, Brouwer KC, Kumar N, Mutapi F, Woelk G, Mduluza T. Efficacy and side effects of praziquantel treatment against *Schistosoma haematobium* infection among primary school children in Zimbabwe. *Trans R Soc Trop Med Hyg* 2008; 102:759–766.

15. Walz Y, Wegmann M, Dech S, Raso G, Utzinger J. Risk profiling of schistosomiasis using remote sensing: approaches, challenges and outlook. *Parasite Vectors* 2015; 8(1):1.

16. World Health Organization. *Schistosomiasis. Status of Schistosomiasis endemic countries*. Geneva: World Health Organization; 2012.

17. World Health Organization. *The control of schistosomiasis. Second report of the WHO expert committee*. Geneva: WHO Technical Report Series; 2014.

18. Hassan AO, Amoo AO, Akinwale OP, Adeleke MA, Gyang PV. Molecular characterization and detection of infection in vector snails of urinary schistosomiasis around Erinle and Eko-Ende Dams in south west Nigeria. *Br Microbiol Res J* 2016; 14(1):1–10.

19. Hassan AO, Amoo AO, Akinwale OP, Deji-Agboola AM, Adeleke MA, Gyang PV. Current status of urinary schistosomiasis in communities around the Erinle and Eko-Ende Dams and the implications for schistosomiasis control in Nigeria. *South Afr J Infect Dis* 2014; 29(4):137–140.

20. Gautret P, Cramer JP, Field V, Caumes E, Jensenius M, Gkrania-Klotsas E. Infectious diseases among travelers and migrants in Europe, European travel network. *Euro Surveill* 2012; 2010:17.

21. Bolaji OS, Adeyeba OA, Ojurongbe O, Odewale G, Ukaga CN. Water contact activities and socio-cultural factors on urinary schistosomiasis in rural area of Osun state, Nigeria. *Int J Res Appl Nat Soc. Sci* 2014; 2(4):101–106.

22. Verweij JJ, ten Hove R, Brienen EA, van Lieshout L. Multiplex detection of *Enterocytozoon bieneusi* and *Encephalitozoon spp*. in fecal samples using gel electrophoresis and real-time PCR. *Diag Microbiol Infect Dis* 2007b; 57:163–167.

23. ten Hove RJ, Verweij JJ, Vereecken K, Polman K, Dieye L, van Lieshout L. Multiplex gel electrophoresis and real-time PCR for the detection and quantification of *Schistosoma mansoni* and *S. haematobium* infection in stool samples collected in northern Senegal. *Trans R Soc Trop Med Hyg* 2008; 102:179–185.

24. Gomes LI, Enk MJ, Rabello A. Diagnosing Schistosomiasis: where are we? *Rev Soc Bras Med Trop* 2014; 47(1):3–11.

25. Cheesborough M. *District laboratory practice in tropical countries*. 2nd ed. Cambridge: Cambridge University Press; 2005.

26. Baiocco P, Gourlay LJ, Angelucci F, Fontaine J, Herve M, Miele AE, Trottle F, Brunori M, Bellelli A. Probing the mechanism of GSH activation in schistosoma haematobium glutathione-S-transferase by site-directed mutagenesis and X-ray crystallography. *J Mol Biol* 2006; 360:678–689.

27. Anosike JC, Nwoke BE, Njoku AJ. The validity of haematuria in the community diagnosis of urinary schistosomiasis infections. *J Helminthol* 2001; 75:223–225.

28. Uneke C, Ugwuok-Adibua S, Nwakpu K, Ngwu B. An assessment of Schistosoma haematobium infection and Urinary tract bacterial infection amongst school children in rural eastern Nigeria. *Internet J Lab Med* 2010; 4:1.

29. Mazigo HD, Nuwaha F, Kinung’hi SM, Morona D, Pinot de Moira A, Wilson S, Heukelbach J, Dunne DW. Epidemiology and control of human schistosomiasis in Tanzania. *J Parasite Vectors* 2012; 5:247.

30. Akinwale O, Ajayi M, Akande D, Adeleke M, Gyag P, Adeneaye A. Prevalence of *Schistosoma haematobium* infection in a neglected community, South Western, Nigeria. *Int J Health Res* 2009; 2:2.

31. Palmer BF. The renal tubule in the progression of chronic renal failure. *J Invest Med* 2007; 45:346–361.

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