



Research article

Association of genetic polymorphism at tumor necrosis factor- α gene promoter - 1031T/C and parasitic infections among children in Northern South AfricaN.C. Davhana^a, A.K. ElBakri^{b,*}, P.O. Bessong^a, A. Samie^a^a Molecular Parasitology and Opportunistic Infection Program, Department of Microbiology, University of Venda, Private Bag X5050, Thohoyandou, 0950, South Africa^b Department of Medical Laboratory Sciences, College of Health Sciences, University of Sharjah, PO Box 27272, Sharjah, United Arab Emirates

ARTICLE INFO

Keywords:

Genetics
Immunology
Microbiology
Molecular biology
TNF- α
PCR-RFLP
Dzimauli community
Limpopo province
South Africa

ABSTRACT

Intestinal parasitic diseases are common in developing countries including South Africa and have been documented to be the most common in children under the age of five. The present study aimed to identify any potential association that may exist between TNF- α promoter gene polymorphism and parasitic infections. A total of 199 blood samples were evaluated from children who were part of the MAL-ED study cohort. The DNA was used to investigate polymorphism in the promoter region of the TNF- α gene at position -1031T/C. The polymorphisms were detected by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay. The TC genotype at position -1031 was significantly higher in healthy controls children than in children who were infected with *Entamoeba* species (59.9% vs 29.4%, $P = 0.015$) and *Entamoeba coli* (59.1% vs 30.8%, $P = 0.046$), indicating that TC genotype may be protective against *Entamoeba* infections and *Entamoeba coli* infections. The CC genotype at position -1031 was more common among children with parasite and diarrhea and the results was statistically significant ($P = 0.04$). This study has revealed that the CC genotype may be a risk factor for symptomatic parasitic infections while the TC genotype might be protective of *Entamoeba* infections among children in Dzimauli community.

1. Introduction

Gastrointestinal parasitic diseases are among the most prevalent infections worldwide, especially in children younger than five years (Ul-Wadood et al., 2005). It is estimated that intestinal parasites affect around 3.5 billion people, particularly in areas of poor sanitation, inadequate water supply and poor hygiene practices (Pullan et al., 2014). *Entamoeba* species, *Cryptosporidium parvum*, *Giardia lamblia*, *Cyclospora* and *Balantidium coli* are among few of the orofecally and/or zoonotically transmitted intestinal parasites that have been documented to be the most common in children under the age of five (Ahmed et al., 2018). Most of these protozoan parasitic infections are generally asymptomatic with only a few manifesting into acute and/or chronic infections. However, despite significant efforts, the precise factors determining the outcome of these infections still remain unknown. Nevertheless, diarrhea is one of the main manifestations of these illnesses (Fischer Walker et al., 2012).

A multifunctional proinflammatory cytokine, TNF- α plays a crucial role in the induction and adaptation of the immune system. It activates inflammatory white blood cells, recruits neutrophils and promotes macrophages to produce other cytokines, such as interleukin (IL)-1 and IL-6 leading to tissue inflammation (Lee et al., 2002; Derouich-Guergour et al., 2001). It can also increase cell permeability, resulting in impairment of barrier function and edema formation (Durmaz et al., 1998). Furthermore, TNF- α is an important inflammatory cytokine involved in the development of resistance to dissimilar microbes including protozoa (Burns et al., 1996; Lean et al., 2006).

Significant genetic variation and polymorphism has been reported in TNF- α , mainly in the promoter region responsible for the transcriptional regulation of the TNF- α gene. Elevated levels of the cytokine have been observed in certain gastrointestinal tract illnesses such as in the case of inflammatory colitis and inflammatory bowel disease (IBD) (Ekhlas et al., 2013). Furthermore, administration of anti-TNF agents has been proven to be effective treatment options for some individuals (Enriquez and Michael, 1998). To our knowledge, not many studies have

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examined the association between genetic polymorphisms at the TNF- α promoter regions and parasitic infections. Thus, in this study, we aimed to investigate whether one of the common polymorphisms in the promoter region of TNF- α (1031T/C) is associated with gastrointestinal tract parasitic infections among children under the aged of five in Northern South Africa.

2. Materials and methods

2.1. Ethical considerations

The present study was part of the MalEd study in the South African site which has been previously described (Bessong et al., 2014). The study was approved by the research and ethics committee of the University of Venda and the Department of Health and Welfare in Polokwane, Limpopo province, South Africa. The objectives of the study were clearly explained to the participants and those who agreed to participate in the research were requested to sign consent forms and complete the questionnaires in order to obtain socio-demographic information. These tools have been fairly described in previous publications (Houpt et al., 2014; Richard et al., 2014). Confidentiality of the participants was kept by giving to each a code. The information obtained from the participants was kept confidential.

2.1.1. Study sites

The study was conducted in the Dzimauli community under Mutale Municipality, Vhembe District, Limpopo Province which is comprised of 9 settlements aligned linearly and adjacent to one another. It is the site of the MalEd study in the South African site previously described (Bessong et al., 2014). The areas are rural with people of different religious, educational and socio-economic backgrounds, living in neighborhoods with distinctly different level of sanitation. Five of the nine settlements (Tshibvumo, Tshapasha, Thongwe, Matshavhawe and Pile) have been identified as the primary areas of the study for the Enteric Infections and Malnutrition (Mal-Ed) project in Limpopo, South Africa.

2.2. Sample collection, processing and genomic DNA extraction

We evaluated 199 blood samples from children who were part of the MAL-ED study cohort. Each sample was labeled with the patient's code, sex, age and the collection date and taken to the University of Venda microbiology laboratory for analysis. Upon arrival all samples were kept on the bench overnight for separation of plasma, buffy coat and red blood cells. DNA was extracted from blood buffy coat using Gen-Elute™ blood genomic kit from Sigma Aldrich following instructions from the manufacturer. Briefly 200 μ l of proteinase K solution was placed into 1.5ml micro-centrifuge tubes where 200 μ l of blood buffy coat was added. 200 μ l of lysis solution C was added to the sample which was vortexed for 15 s. The sample mixture was then incubated at 55 °C for 10 min. The samples were prepared for binding by adding 200 μ l of ethanol to the mixture and vortexed again for 10 s. The entire mixture was transferred to a column which was first prepared by addition of 500 μ l of column preparation solution and centrifuged at 12000 rpm for 1 min. The columns were then washed twice with the addition of wash solutions, and centrifuged for 3 min at maximum speed. The DNA was then eluted by pipetting a 200 μ l of elution solution into the center of the column directly and centrifuged for 1 min at maximum speed. The DNA was then stored in the freezer at -20⁰C until further analysis.

Stool samples from the participants were tested for parasites using parasitological methods as previously described (Platts-Mills et al., 2014).

2.3. PCR-RFLP analysis of TNF- α

Genomic DNA was used to investigate the polymorphism in the promoter region of the TNF- α gene at position -1031T/C using polymerase chain reaction–restriction fragment length polymorphism

(PCR–RFLP) assay as previously described by Naderi et al. (2014). Genotyping for the -1031T/C was performed using a PCR fragment amplified by the forward (5'-GGGGAGAACAAAAGGATAAG-3') and reverse (5'-CCCCATACTCGACTTTCATA-3') primer pairs. Briefly, a total of 30 cycles was carried out; each of these consisted of 95 °C for 30s, 55 °C for 30s, 72 °C for 30s. There was an initial denaturation step at 95 °C for 5 min and a final extension step at 72 °C for 5 min. The 1031T/C polymorphism (C allele, 180 + 71+13bp; T allele, 251 + 13bp) was later evaluated by digestion with the restriction enzyme BbsI as described by the manufacturer (New England Biolabs, Ipswich MA, USA) at 37 °C for 16h. Digested PCR fragments were separated by a 4% of agarose gel electrophoresis and visualized by ethidium bromide staining.

2.4. Statistical analyses

Statistical analysis was performed using the chi-square test to determine statistical associations between the genotype distribution and the occurrence of parasitic infections. P-value less than 0.05 were considered statistically significant. Odds ratio (OR) and 95% confidence intervals (CI) were also calculated. All data analysis was conducted using SPSS software, version 24.

3. Results

3.1. The overall demographic and clinical characteristics of the study population

A total of 199 blood samples were collected from 199 participants who were part of the MalEd study (The MAL-ED Network Investigators, 2014), of which 94 (47.2%) were males and 105 (52.8%) were females. Table 1 summarizes the demographic data of the study population. Of the 199 blood samples collected, 182 (91.5%) never had any *Entamoeba species* while 17 (8.5%) had *Entamoeba species*. From the study population, about 98 (49.2%) participants were found to had been infected with *Giardia lamblia* followed by *Cryptosporidium* with 56 (28.1%), *Entamoeba coli* with 13 (6.5%), *Balantidium* with 12 (6.0%). Of all children, only 1 (0.5%) was found to had infected with *Cyclospora* (Figure 1).

3.2. Prevalence of parasite and infection in the study population

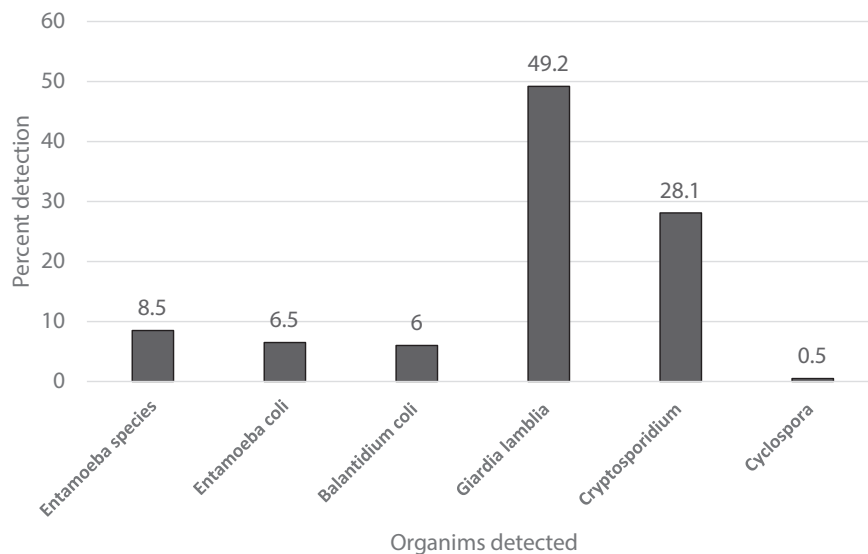
Of the total number of participants, 27.6% had been infected with parasite and exhibited acute lower respiratory tract infection while 72.4% participant had no parasite infection nor had shown acute lower respiratory infection. On the other hand, 54.3% of the children never had been infected with parasite and nor had diarrhea whereas 45.7% had had infections with a parasite with diarrhea. Of the 199 children, only 80 (40.2%) were found to had been infected with parasite and exhibited illness except 59.8% had never been infected with parasite and illness.

3.3. Association of TNF- α -1031T/C polymorphism with *Entamoeba species*, *Entamoeba coli*, *Cryptosporidium parvum*, *Giardia lamblia*, *Balantidium coli* and *Cyclospora*

Table 3 illustrates the association between TNF- α -1031T/C polymorphism, *Entamoeba species*, *Entamoeba coli*, *Cryptosporidium parvum*, *Giardia lamblia*, *Balantidium coli* and *Cyclospora*. The CC and TT genotypes at position -1031T/C were more common among children with *Entamoeba species* than in the control group even though the results were insignificant (P = 0.106, X² = 2.606, OR = 2.640, 95% CI = 0.781–8.916) and (P = 0.139, X² = 2.192, OR = 2.107, 95% CI = 0.772–5.751); respectively. On the other hand, TC genotype was common and statistically significant in children who had no *Entamoeba species* (P = 0.015, X² = 5.902, OR = 0.279, 95% CI = 0.094–1.825). Similarly, both the CC and TT genotypes was more common among children with *Entamoeba coli* than the control group with the results being statistically insignificant (P = 0.179, X² = 1.805, OR = 2.490, 95% CI =

Table 1. Demographic and clinical features of the study population.

| Characteristics | | Frequency | Percentage (%) |
|--|--|-----------|----------------|
| Gender | Male | 94 | 47.2% |
| | Female | 105 | 52.8% |
| Low birth weight | Never had low birth weight | 176 | 88.4 |
| | Had low BWT | 23 | 11.6 |
| High birth weight | Never had high weight | 159 | 79.9 |
| | Had high birth weight | 40 | 20.1 |
| Colostrum | No colostrum | 7 | 3.5 |
| | Colostrum | 192 | 96.5 |
| Diarrhea | Had no diarrhea at all | 75 | 37.7 |
| | Had had at least one diarrheal episode | 124 | 62.3 |
| Vomit | Never had any vomiting episode | 177 | 88.9 |
| | Had had at least one episode of Vomiting | 22 | 11.1 |
| Fever | Never had any fever episode | 176 | 88.4 |
| | Had had at least one fever episode | 23 | 11.6 |
| Acute lower respiratory infection (ALRI) | Never had an ALRI episode | 120 | 60.3 |
| | Had had at least one ALRI episode | 79 | 39.7 |

**Figure 1.** Occurrence of some parasitic organisms in the study population.

0.632–9.810) and ($P = 0.227$, $X = 1.459$, $OR = 1.990$, $95\% CI = 0.772–5.751$). However, the TC genotype was frequent and statistically significant in children who had no *Entamoeba coli* ($P = 0.046$, $X^2 = 3.997$, $OR = 0.307$, $95\% CI = 0.091–1.033$). On the other hand, the CC genotype noted to have been more frequent and statistically significant among children with *Cyclospora* infections $P = 0.006$, $X^2 = 7.691$, $OR = 1.045$, $95\% CI = 0.958–1.141$) (Table 2).

3.4. Association of TNF- α -1031T/C polymorphism with parasite and diarrhea, parasite and acute lower respiratory infection, and parasite and illness

Table 3 illustrates the association between TNF- α -1031T/C polymorphism and the overall parasite and diarrhea, acute lower respiratory infection, and illness. The CC genotype at position -1031T/C was statistically significant among children with overall parasite and diarrhea ($P = 0.04$, $X^2 = 8.324$, $OR = 3.905$, $95\% CI = 1.469–10.381$), with overall parasite and acute lower respiratory infection ($P = 0.005$, $X^2 = 7.828$, $OR = 3.374$, $95\% CI = 1.389–8.196$), and those with overall parasite and illness ($P = 0.032$, $X^2 = 4.621$, $OR = 2.593$, $95\% CI = 1.063–6.321$). On the other hand, TT genotype was significantly abundant in healthy

individuals (37.0%) than children who had parasite and illness (22.5%) ($P = 0.031$, $X^2 = 4.673$, $OR = 0.495$, $95\% CI = 0.260–0.942$) (Table 3).

4. Discussion

Enteric protozoan parasites like *Entamoeba histolytica*, *Cryptosporidium parvum* and *Giardia lamblia* are regarded as a serious public health problem and important causes of diarrheal disease (Xiao, 2010; Yaoyu and Xiao, 2011). Millions of people are annually infected with *E. histolytica* and *G. lamblia*, making the diseases a major cause of morbidity worldwide (Teles et al., 2011). Cytokines play important roles in the activation and regulation of human immune responses and their production are variable due to single nucleotide polymorphisms (Ollier, 2004). No evidence on the association between TNF- α and parasitic infections in Limpopo Province of South Africa, particularly in the rural areas. Furthermore, the role of host genetic factors that might contribute to the burden of diseases has never been studied especially in districts within the Limpopo Province. Therefore, there is a need to identify any potential association that may exist between TNF- α promoter gene polymorphisms and parasitic infections.

Of the study participants (ages 0–5 years) with and without diarrhea recruited in this analysis, 28.1% were positive for *Cryptosporidium* species.

Table 2. TNF- α genotype at position -1031T/C in patients with *Entamoeba species*, *Entamoeba coli*, *Cryptosporidium parvum*, *Giardia lamblia*, *Balantidium* and *Cyclospora*.

| | | Did not have the infection | Had the infection | Significance | OR | 95% CI |
|-------------------------------|-----------|----------------------------|-------------------|--|--------------|--------------------|
| <i>Entamoeba spp</i> | CC | 19 (10.4%) | 4 (23.5%) | P = 0.106 ($\chi^2 = 2.606$) | 2.640 | 0.781–8.916 |
| | TC | 109(59.9%) | 5(29.4%) | P = 0.015 ($\chi^2 = 5.902$) | 0.279 | 0.094–1.825 |
| | TT | 54 (29.7%) | 8 (47.1%) | P = 0.139 ($\chi^2 = 2.192$) | 2.107 | 0.772–5.751 |
| <i>Entamoeba coli</i> | CC | 20 (10.8%) | 3 (23.1%) | P = 0.179 ($\chi^2 = 1.805$) | 2.490 | 0.632–9.810 |
| | TC | 110(59.1%) | 4(30.8%) | P = 0.046 ($\chi^2 = 3.997$) | 0.307 | 0.091–1.033 |
| | TT | 56 (30.1%) | 6 (46.2%) | P = 0.227 ($\chi^2 = 1.459$) | 1.990 | 0.640–6.188 |
| <i>Giardia lamblia</i> | CC | 10 (9.9%) | 13 (13.3%) | P = 0.458 ($\chi^2 = 0.551$) | 1.392 | 0.580–3.341 |
| | TC | 57 (56.4%) | 57 (58.2%) | P = 0.061 ($\chi^2 = 805$) | 1.073 | 0.612–1.882 |
| | TT | 34 (33.7%) | 28 (28.6%) | P = 0.438 ($\chi^2 = 601$) | 0.788 | 0.432–1.439 |
| <i>Balantidium coli</i> | CC | 20 (10.7%) | 3 (25.0%) | P = 0.133 ($\chi^2 = 2.257$) | 2.783 | 0.696–11.136 |
| | TC | 108 (57.8%) | 6 (25.0%) | P = 0.277 ($\chi^2 = 0.599$) | 0.731 | 0.227–2.353 |
| | TT | 59 (31.6%) | 3 (25.0%) | P = 0.226 ($\chi^2 = 0.635$) | 0.723 | 0.189–2.769 |
| <i>Cyclospora</i> | CC | 22(11.1%) | 1(100.0%) | P = 0.006 ($\chi^2 = 7.691$) | 1.045 | 0.958–1.141 |
| | TC | 114 (57.6%) | 0 (0.0%) | P = 0.246 ($\chi^2 = 1.348$) | 0.988 | 0.966–1.011 |
| | TT | 62 (31.3%) | 0 (0.0%) | P = 0.455 ($\chi^2 = 0.500$) | 0.993 | 0.979–1.007 |
| <i>Cryptosporidium parvum</i> | CC | 16 (11.2%) | 7 (12.5%) | P = 0.068 ($\chi^2 = 0.795$) | 1.134 | 0.440–2.924 |
| | TC | 78 (54.5%) | 36 (64.3%) | P = 0.212 ($\chi^2 = 1.560$) | 1.500 | 0.792–2.839 |
| | TT | 49 (34.3%) | 13 (23.2%) | P = 0.130 ($\chi^2 = 2.291$) | 0.580 | 0.285–1.180 |

The results were found significant if the P value was less than 0.05 and are indicated in bold.

This result is higher than those reported from Ethiopia and Tanzania with 9.4% and 10.4% positive rates, respectively (Kabayiza et al., 2014 and Nasser, 2016). The current study revealed a prevalence of 49.2% with *Giardia lamblia* among children less than 5 years. This is unlike a report by Naz et al. (2018), in which they observed a much less prevalence (11.1%) among children of the same age range as those in the current survey. On the other hand, a much lower prevalence of *Cyclospora* (0.5%) was determined in the study group unlike a Nepalese survey where a 7.9% was reported among diarrheal children (Sherchan et al., 2010).

The present study also examined any possible association between TNF- α promoter gene polymorphism at position -1031T/C with parasitic infections. Our study found an association between differences in the TNF- α promoter gene polymorphism at position -1031T/C and susceptibility to the development of parasitic diseases leading us to postulate that these polymorphic differences might play an important role in the pathophysiology of these parasitic diseases.

Previous studies have shown conflicting results regarding the role of TNF- α and amoebae. In the present study, the TC genotype was significantly associated with reduced prevalence of *Entamoeba* species (p = 0.015). Indicating that the TC genotype may be protective against *Entamoeba* infections. Some *in vitro* studies have revealed that TNF- α leads to increased nitric oxide (NO) production and killing of amoeba (Séguin et al., 1997). These findings would suggest that

TNF- α may be beneficial in amoebic colitis. In this study, heterozygous TC genotype of the SNP -1031T/C was significantly higher in healthy control than children with *Entamoeba species* and *Entamoeba coli*. This indicates that -1031T/C genotype may offer a protective effect against *Entamoeba species* and *Entamoeba coli*. TNF- α has been shown to worsen tissue damage in mouse models of amoebic colitis (Zhang et al., 2003 and Zhang and Stanley, 2004). The TNF- α also attracts *E. histolytica* trophozoites through chemotaxis (Blazquez et al., 2006), which may aid the parasite in the process of tissue invasion and colitis. Although not statistically significant, the present study showed that the CC genotype of the SNP -1031T/C was greater in children with *Giardia lamblia* as compared to healthy control. Zhou et al. (2007) showed that TNF- α plays an important role in the early control of giardiasis.

Our results also showed that homozygous CC genotype of the SNP -1031T/C was significantly higher among children with *Cyclospora* and overall parasitic infection with diarrhea and acute lower respiratory infection, indicating that CC genotype may be a factor for *Cyclospora*. These findings are in agreement with a previous study by Akman et al. (2006), who showed that TNF- α -1031CC genotype was also associated with a significantly higher serum TNF- α level in Behcet's disease. Another study by Sohail et al. (2008), also revealed that TNF- α -1031CC genotype

Table 3. TNF- α genotype at position -1031T/C in patients with parasite and diarrhea, parasite and acute lower respiratory infection, and parasite and illness.

| Characteristics | | Did not have the condition | Those that had the condition | Significance | OR | 95% CI |
|--|----|----------------------------|------------------------------|--|--------------|---------------------|
| Parasite and diarrhea | CC | 6 (5.6%) | 17 (18.7%) | P = 0.04 ($\chi^2 = 8.324$) | 3.905 | 1.469–10.381 |
| | TC | 63 (58.3%) | 51 (56.0%) | P = 0.106 ($\chi^2 = 0.745$) | 0.911 | 0.518–1.600 |
| | TT | 39 (36.1%) | 23 (25.3%) | P = 0.100 ($\chi^2 = 2.704$) | 0.598 | 0.324–1.106 |
| Parasite and acute lower respiratory infection | CC | 11(7.6%) | 12(21.8%) | P = 0.005 ($\chi^2 = 7.828$) | 3.374 | 1.389–8.196 |
| | TC | 85 (59.0%) | 29 (52.7%) | P = 0.422 ($\chi^2 = 0.646$) | 0.774 | 0.414–1.446 |
| | TT | 48 (33.3%) | 14 (25.5%) | P = 0.283 ($\chi^2 = 1.152$) | 0.683 | 0.340–1.373 |
| Parasite and any illness | CC | 9(7.6%) | 14(17.5%) | P = 0.032 ($\chi^2 = 4.621$) | 2.593 | 1.063–6.321 |
| | TC | 66 (55.5%) | 48 (60.0%) | P = 0.403 ($\chi^2 = 0.526$) | 1.205 | 0.678–2.141 |
| | TT | 44(37.0%) | 18(22.5%) | P = 0.031 ($\chi^2 = 4.673$) | 0.495 | 0.260–0.942 |

Note: Illness here represents any type of illness identified in the child at the time of sample collection including any of the following: Diarrhea, Acute lower respiratory syndrome, Vomiting and Fever.

The results were found significant if the P value was less than 0.05 and are indicated in bold.

was also associated with a significantly higher serum level of TNF- α in malaria patients.

Although no significant association was observed, heterozygous TC genotype of the SNP -1031 was greater among the children with *Cryptosporidium* infections as opposed to the control group. A previous study carried out by Nourian et al. (2017), demonstrated similar results with regards to the TC genotype which increased in patients with IBD in an Iranian population nonetheless no significant association was discerned.

In conclusion, the present study shows for the first time that -1031 (T/C) polymorphism of the TNF- α promoter gene is associated with pathogenic parasitic infections, particularly *Entamoeba* spp and *Cyclospora* infections. In particular, the heterozygote genotypes were associated with protection against *Entamoeba* infections while the homozygote genotypes seemed to be more common among participants with more infections. However, this polymorphism of TNF- α promoter gene was not associated with *Cryptosporidium* species, *Giardia lamblia* and *Balantidium coli*, suggesting that polymorphism might not have an impact on the occurrence of these infections among children.

Declarations

Author contribution statement

Davhana NC: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

ElBakri AK: Analyzed and interpreted the data.

Bessong PO, Samie A: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Funding statement

This work was supported by a grant made available to Prof. Samie, A through the National Research Foundation of South Africa (Grant number IFR180215313776). The study was also funded by University of Virginia with support from MAL-ED Network Investigators from the Foundation of National Institute of Health, Fogarty International Center (FIC) with overall support from the Bill & Melinda Gates Foundation.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors would like to thank the hospital management and the patients for their cooperation throughout the study.

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