ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 6 No. 3 September 2020*

Evaluation of Effective Performances of Three Parasitological Procedures in Detection of Intestinal Parasitic Infections

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Abstract

Parasitological diagnostic methods are useful for detecting intestinal parasites through searching for different parasitic forms (e.g. eggs, larvae, oocysts or trophozoites) that are eliminated in feces. Despite improvements in immunological techniques and the advent of molecular tests, parasitological methods are still considered important, particularly because of their simplicity and low cost.This study was aimed at evaluating the performance of the three parasitological procedures; direct wet mount, Concentration technique and Modified Zeihl Neelsen staining technique used in detection of intestinal parasite infections. This hospital-based prospective study was conducted in three selected hospitals in Kano state Viz: Gaya General Hospital, Infectious Diseases Hospital and Murtala Muhammad Specialists Hospital. A total of 330 HIV positive patients aged 18 and above years of both sexes, were involved in the study from January 2016 to May 2017. Stool samples were collected from the participants and examined using direct wet mount, formol-ether concentration method and modified Ziehl-Neelsen (ZN) staining method. Data was analysed using SPSS version 20 software and p-value was set at $p<0.05$.The overall prevalence of intestinal parasites detected by the formol-ether concentration (gold standard) method was 13.9%. The direct wet mount and modified Ziehl Neelsen methods gave overall prevalence of 6.1% and 6.7% respectively. The evaluation results of the performances of direct wet mount and modified Ziehl Neelsen methods against the formol-ether

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concentration (gold standard) method gave sensitivity and specificity as 43.5% (20/46) and 100% (284/284) and 47.8% (22/46) and 100% (284/284) respectively. This study has indicated the value of using various parasitological procedures in the analysis of fecal specimens for intestinal parasitic infections. The study also gives room for investigating whether our clinical laboratories have enough resources (staff and equipment) to apply multiples parasitological procedures in yielding the diagnosing of intestinal parasitic infections.

Keywords: Intestinal Parasite, Modified Zeihl Neelsen staining,

INTRODUCTION

Parasites infections significantly contribute to the burden of gastrointestinal illness worldwide (Fletcher *et al.,* 2012). Approximately 100 species of animal parasites can infect the body and about 70 of the parasites are common and considered important. More than half of these parasites can be detected by examination of fecal specimens because they reside in the gastrointestinal tract itself or are so located that they or their progeny finds their way into the alimentary canal (WHO, 2012).

Definitive diagnosis of parasitic infections depends on demonstration of a stage of the parasite's life cycle in the human host (Garcia *et al.,* 2000). The adult worms, that inhabit the intestine, discharge the eggs or larvae they produce in faeces (Neva and Brown, 1994). Therefore, laboratory diagnosis of intestinal parasites is based on detection and identification of characteristic eggs or larvae in stool samples (Parija and Srinivasa, 1999). A wide variety of laboratory methods, including parasitological, molecular, serologic and cultural approaches, have been developed over the years for diagnosis of intestinal parasites (Markell *et al.,* 1999).

Parasitological diagnostic methods are useful for detecting intestinal parasites through searching for different parasitic forms (e.g. eggs, larvae, oocysts or trophozoites) that are eliminated in feces. Despite improvements in immunological techniques and the advent of molecular tests, parasitological methods are still considered important, particularly because of their simplicity and low cost (Nunez *et al*., 1999; Papini *et al*., 2012). However, since the diagnosis is based on viewing eggs or oocysts, confirmation may in some cases be difficult (Rey, 2008). In addition, choosing the most suitable method for use within the routine of the diagnostic laboratory may represent an obstacle for efficient diagnosis.

The macroscopic appearance of stool specimen can give a clue to the type of organisms present (Goodman *et al.,* 2007).Microscopic or parasitologic diagnosis is generally sensitive, simple, and economical (Parija and Srinivasa, 1999). If performed correctly, stool microscopy offers many advantages over other diagnostic methods for detecting intestinal parasites (Bogoch *et al.,* 2006).

MATERIALS AND METHODS

Study Area

The study was carried out at some selected hospitals in Kano State, Nigeria which comprise of Murtala Muhammad Hospital, Gaya General Hospital, and Infectious Diseases Hospital (IDH) all in Kano, Nigeria.

Study Design and Study Population
A prospective cross-sectional hospital based study was carried. All consented adult patients living with HIV that attended AKTH.

Sample Collection
A total of 330 stool samples were collected from adult confirmed HIV clients in sterile wide mouth screw container and labeled. The consented participants were directed to collect about 10gram of stool samples, how to collect the samples and delivered it within 2 hours. All the samples were analyzed within 24hours of collection (Morris *et al*., 1992).

Sample Processing
Three parasitological methods were used for the purpose of this study, namely the direct wet mount, formol-ether concentration and modified Ziehl Neelsen (ZN) staining method. Each specimen was first examined macroscopically and its consistency or nature was recorded as either formed (F), semi-formed (SF), semi-formed with blood (SB), bloody-mucoid (BM), loose (L) or watery (W), in accordance with the description by Cheesbrough (2009). Samples were analyzed fresh, in batches, as soon as they were received; none was preserved in the refrigerator or any preservative added prior to processing, as this would kill larvae or motileparasites which may be present (Smith and Schad, 1990).The test procedures were carried out in accordance with standard protocols as described by WHO (2006).

Direct Saline Wet Mount Method
A single stool sample was obtained in labeled specimen containers from all consenting patients selected for the study. A direct saline mount of each sample was prepared and microscopically checked for motile intestinal parasites. Lugol's iodine staining was done, after the stained concentrate was well mixed using a sterile Pasteur pipette. A drop was placed on a labeled clean dry 76mm x 26mm slide and covered with a 20mm x 20mm cover glass. The preparation was examined with Olympus light microscope using the low power (X10) and the high power (X40) objectives for helminth larvae and ova identification. The x10 and x40 objectives were used for searching and identification of the cysts and trophozoites of protozoa respectively. The number of cyst, ova, larvae or parasite density level was estimated as follows: Scanty = 1-3 parasites per preparation, Few = 4-10 parasites per preparation, Moderate = 11-20 parasites per preparation, Many = 21-40, Very many = >40 parasites per preparation (Cheesbrough, 2009).

Formol-Ether Concentration Technique for Stool Examination
The modified formal – ether concentration method by sedimentation technique as described by Cheesbrough (2009) was used for individuals with light infections that could otherwise not be detected by direct saline wet mount. With the aid of an applicator stick, about1g of each fresh stool sample was emulsified in 3-4ml of 10% formalin and the content transferred into 10ml centrifuge tube. The content was mixed by shaking for 20 seconds and then sieved with double layer cotton gauze, collecting the sieved suspension in a beaker. The sieved suspension was poured back into the centrifuge tube and the debris discarded. Equal volume of ether (3-4ml) was added, mixed well and the content centrifuged at 3000 rpm for 1 minute. The supernatant was decanted and the tube placed in a rack. The sediment was transferred onto a slide, stained with iodine, covered with a coverslip and examined for the presence of ova or parasites under the light microscope at a magnification of X10 and X40 (Cheesbrough, 2009).

Modified Zeihl Neelsen (ZN) staining Technique

A small portion of the fresh stool sample was processed for Cryptosporidium parvum and microsporidia oocysts using the Ziehl-Neelsen (ZN) method with some modification. Thin smear was prepared directly from fresh stool as well as from sediment of concentrated stool and allowed to air dry. The slides were then fixed with methanol for 5minutes and stained with carbol fuchsin for 30minutes. After washing the slides in tap- water, they were decolourised with acid alcohol (99ml of 96% ethanol and 1ml HCl) for 1-3 minutes and counterstained in methylene blue for one minute. The slides were then washed in tap water and observed under light microscope with a magnification of X100. Oocysts for Cryptosporidium appeared bright orange with clear halo against a blue background, measuring 4-6µm in size. For microsporidium the spores were very small measuring 1.0-1.7µm in size (Cheesbrough, 2009).

RESULTS

The overall prevalence of intestinal parasites detected by the formol-ether concentration method was 13.9%, while direct wet mount and modified Ziehl-neelsen methods gave overall prevalence of 6.1% and 6.7% respectively (Table 1). The performance of direct wet mount was evaluated in relation to formol-ether concentration which was the gold standard test and the evaluation results are shown in Table 2. The wet mount method detected a total of 20 intestinal parasites as against 46 by the formol-ether concentration method (gold standard). The evaluation results gave sensitivity and specificity of the wet mount method as 43.5% (20/46) and 100% (284/284), respectively. The performance of the modified Ziehl-Neelsen staining method was compared with the formol-ether concentration method results which gave a sensitivity of 47.8% (22/46) (Table 3). The modified Ziehl-Neelsen (ZN) staining method was good in detecting *Cryptosporidium* and *Microsporidium* spp in this study.

Table 1: prevalence of intestinal parasites stratified by the parasitological methods used

Parasite	Direct Wet Mount n(%)	Formol-ether Conc n(%)	Modified Ziehl-Neelsen n(%)
Entamoeba histolytica	12 (3.6)	23 (7.0)	0 (0.0)
Entamoeba coli	1 (0.3)	7 (2.1)	0 (0.0)
Giardia lamblia	4 (1.2)	9 (2.7)	0 (0.0)
Hymenolepsis nana	1 (0.3)	1 (0.3)	0 (0.0)
Cryptosporidium. Parvum	0 (0.0)	2 (0.6)	10 (3.0)
Microsporidium	0 (0.0)	1 (0.3)	7 (2.1)
Hookworm	0 (0.0)	3 (0.9)	0 (0.0)
Mix infection	2 (0.6)	0 (0.0)	5 (1.5)
Total	20 (6.1)	46 (13.9)	22 (6.7)

Table 2: Performance of Direct Wet Mount against Formol-Ether Concentration Method

Formol-ether Concentration	Direct wet mount test		Total
	Positive	Negative	
Positive	20	26	46
Negative	0	284	284
Total	**20**	**310**	**330**

Positive predictive value, PPV = 43.5% (20/46)

Table 3: Performance of modified ziehl-neelsen staining method against formol-ether concentration method

Formol-ether Concentration	Modified Zeihl Neelsen		Total
	Positive	Negative	
Positive	22	24	46
Negative	0	284	284
Total	**22**	**308**	**330**

Positive predictive value, PPV = 47.8% (22/46)

DISSCUSION

The overall prevalence of intestinal parasite infections as detected by the formol-ether concentration (which was used as the gold standard method for the study), direct wet mount and modified Zeihl Neelseen (ZN) methods were 13.9%, 6.1%and 6.7%, respectively (Table 1). The prevalence of opportunistic intestinal parasites detected by the modified Ziehl Neelsen (ZN) staining technique was 10.2%. Analysis of the diagnostic performance of direct wet mount method and modified Ziehl Neelsen (ZN) staining technique gave sensitivities of 43.5%, and 47.8% respectively. It was observed that, direct wet mount and modified Ziehl Neelsen (ZN) staining technique were better in the detection of intestinal protozoan parasites, intestinal and opportunistic intestinal parasites respectively.

The study has revealed that, the direct wet mount was as good as the gold standard for the detection of intestinal protozoan parasites such as *G. lamblia* and *E. histolytica*. Similar observation was reported by Watson *et al*. (1988) that the direct wet mount method is useful for detecting organism motility, particularly for the observation of motile trophozoites of intestinal protozoan parasites. Melvin and Brooke (1985) also reported that, the method is also useful for diagnosis of parasites that may be lost in concentration techniques. The overall prevalence rate of 6.1% by the direct wet mount method for intestinal parasites observed in this study could be due to the fact that majority of infected patients have low parasite burden.

The modified Ziehl Neelsen (ZN) staining method was good for the detection of opportunistic intestinal parasites such as Cryptosporidium and Microsporidium spp. A comparison of the performance of the modified Ziehl Neelsen (ZN) staining method and the gold standard results gave sensitivity of 47.8% in this study (Table 4.5). Adamu and Petros (2009) and Assefa *et al*. (2009) also reported the use of the modified Ziehl Neelsen (ZN) staining method to detect *Cryptosporidium* and *Microsporidium* infections in studies conducted in different parts of Ethiopia. It is therefore imperative that this method be used in all parasitological laboratories, especially for the detection and confirmation of opportunistic intestinal parasites in HIV/AIDS patients.

CONCLUSION

This study has indicated the value of using various parasitological procedures in the analysis of fecal specimens for intestinal parasitic infections. The study also gives room for investigating whether our clinical laboratories have enough resources (staff and equipment) to apply multiple parasitological procedures in yielding the diagnosing of intestinal parasitic infections.

The shortfall of all the three methods is that, they could not differentiate *E. histolytica* which is very pathogenic from *E. dispar* which is not pathogenic. Real time PCR and enzyme-linked immunosorbent assay-based kits can be used to differentiate between *E. histolytica* and *E. dispar* (Leo *et al*., 2006).

RECOMMENDATIONS

Based on the results obtained and in order to avoid frequent infection, we recommend that; when faecal samples are to be analyzed, Multiples Parasitological diagnostic techniques should be applied so that all or most of the intestinal parasite infections can be diagnosed especially the opportunistic parasites.

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