Original Article

DOI: 10.7860/JCDR/2022/51015.16120

Microbiology Section

Diagnostic Significance of Wet Mount Microscopy- A Retrospective Observational Study

ABHA SHARMA[1], POONAM SOOD LOMBA[2], BIBHABATI MISHRA[3], ASHNA BHASIN[4], SULMAZ RESHI[5]

ABSTRACT

Introduction: Wet mount microscopy is a rapid and easy conventional technique that provides a quick answer when positive and it provides an approximation of the infection burden. In this era of modern medicine where almost every infection is being diagnosed, using expensive and sophisticated molecular techniques, a simple wet mount examination of a clinical sample can still be relevant in several infections and play a significant role in the early diagnosis and treatment of infectious diseases.

Aim: To focus and review the significance of wet mount examination of clinical specimens for diagnosis.

Materials and Methods: A retrospective observational study with 11 cases was conducted at a superspeciality hospital in New Delhi over a period of six months from March 2019 to August 2019. Direct microscopic demonstration of motile *E. histolytica* trophozoites in saline wet mount of colonic biopsy specimens was done in patients presenting with non specific gastrointestinal symptoms. Positive cases were immediately reported to the clinician for further management. In view of this study, literature search was done on PubMed and Google scholar platforms for studies on wet mount examination of clinical specimens, data was analysed and all infectious diseases were identified for which wet mount microscopy will help in decreasing the Turn around Time (TAT) for diagnosing the infection from 24 hours to 20-30 minutes in real time.

Results: Motile trophozoites of *E. histolytica* were seen in eight out of 11 cases studied. Amoebic serology by Enzyme-linked Immunoassay (ELISA) and histopathology for amoebic trophozoites was positive in all eight patients with 100% association between microscopic demonstrations of motile *E. histolytica* trophozoites in saline wet mount for amoebiasis.

Conclusion: Right test at right time for clinical management of infectious diseases requires good communication between laboratory and clinicians. Direct Wet mount microscopy of clinical samples is rapid, simple, cost-effective method to decrease TAT and guide the clinicians to rule out infections and avoid unnecessary empirical use of antimicrobial drugs.

Keywords: Clinical specimen, Diagnostic stewardship, Gastrointestinal symptoms, Infectious disease

INTRODUCTION

The role of Rapid Diagnostic Tests (RDTs) and point of care tests in clinical microbiology for diagnosis of infectious diseases has been the focus of researchers in recent years and there have been significant developments also. The RDTs have the potential to affect diagnostic algorithms and therapeutic decisions [1]. Many new rapid molecular diagnostic techniques for infectious diseases have been developed that provide faster results enabling rapid delivery of patient care [2]. The appropriate use of laboratory tests to guide patient treatment and optimise clinical outcome is diagnostic stewardship. The ultimate goal is to limit spread of antimicrobial resistance. Diagnostic stewardship is a collaborative effort with team work between laboratory and clinician so as to order the right test at right time and result can be interpretated with less TAT in real time for early patient management [3].

However, it seems that in today's world in order to achieve this goal the clinical laboratories are directly jumping into automation and molecular diagnostic techniques for diagnosing almost all infections. This might be the best approach in the developed countries but in developing countries with limited resources, except for viral infections, the role of old conventional techniques in diagnosis of all other infections should still have a place in clinical diagnostic microbiology. A direct simple wet mount examination of clinical specimen is facing extinction in this era of modern medicine. The aim of this study was to focus and review the significance of wet mount examination of clinical specimens in order to bring the attention of clinical microbiologists towards the role of simple, cost effective laboratory test like wet mount microscopy that can also easily guide in patient management.

MATERIALS AND METHODS

It was a retrospective observational study conducted at a superspeciality hospital in New Delhi over a period of six months from March 2019 to August 2019. After receiving approval from the Institutional Ethical Committee (EC/Micro/03/2/2019/10). The role of direct microscopy in the diagnosis of amoebic colitis was studied and in view of this study, literature search was done on PubMed and Google scholar platforms for studies done on wet mount examination of clinical specimens. Data was analysed and all infectious diseases were identified for which wet mount microscopy will help in decreasing the TAT for diagnosing the infection from 24 hrs to 20-30 minutes in real time.

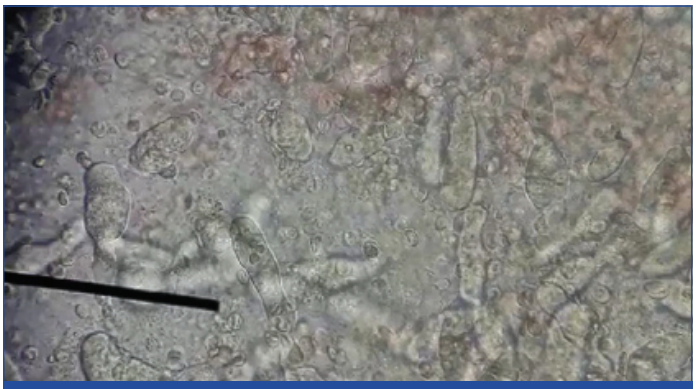
Inclusion criteria: Referred cases presenting to the Gastroeneterology Out-patient Department of a superspecialty hospital with non specific gastrointestinal symptoms and history of bloody diarrhoea, suspected of having chronic amoebic colitis on clinical examination were included in the study. Clinical history was recorded in a proforma.

Exclusion criteria: All patients with no history of diarrhoea and no clinical history suggestive of colitis were excluded from the study.

Study Procedure

Serum sample was collected from all patients for amoebic serology by ELISA. Colonoscopic examination of all patients was perfomed for evidence of diffuse mucosal inflammation, with or without mucosal ulceration. Two colon biopsy specimens were collected during colonoscopy. One was kept in sterile container filled with formalin and sent to the pathology laboratory for histopathological examination for confirmation of invasive amebiasis in colonic tissue.

The other specimen was kept in sterile container filled with normal saline and immediately transferred to the microbiology lab within 15 minutes of taking the biopsy for direct saline wet mount preparation of colonic biopsy specimen. In the microbiology laboratory, a wet mount on glass slide was prepared by taking a small drop of saline on the slide and adding a small amount of biopsy tissue from the container with the help of clean forceps. Then a cover slip was placed over the wet mount preparation on slide and the slide was examined under 400x (high power) of light microscope. Direct microscopic demonstration of motile *E. histolytica* trophozoites in saline wet mount of colonic biopsy specimens was done [Table/Fig-1]. The positive cases were immediately reported to the clinician for further management of the cases.



[Table/Fig-1]: Saline wet mount of colon biopsy showing multiple motile trophozoites of *E.histolytica* seen with ingested RBCs (400X).

STATISTICAL ANALYSIS

Descriptive statistics were used for analysis of the data.

RESULTS

A total of 11 cases were presented and studied (males=7 and females=4) with mean age 52.2 years for females and 25 years for males. Motile trophozoites of *E. histolytica* were seen in eight cases. Amoebic serology by ELISA and histopathology for amoebic trophozoites was positive in all eight patients. There was 100% association between microscopic demonstration of motile *E. histolytica* trophozoites in saline wet mount of colonic biopsy and trophozoites seen in histopathologic examination of colonic biopsy and amoebic serology for serum antibodies for amoebiasis [Table/Fig-2].

Variables		Saline wet mount of colonic biopsy		Kappa value (agreement level)
		Motile trophozoites of *E. histolytica* seen	Motile trophozoites of *E. histolytica* not seen	
Amoebic serology	Positive	8	0	Kappa=1.000 (perfect agreement)
	Negative	0	3	
Histopathology of colonic biopsy	Ameobic trophozoites seen	8	0	Kappa=1.000 (perfect agreement)
	Ameobic trophozoites not seen	0	3	

[Table/Fig-2]: Association of colon biopsy wet mount with histopathology and serology.

DISCUSSION

In order to prevent treatment failure and the development of antimicrobial resistance it is important that early presumptive diagnosis of infectious diseases gain attention by the researchers. For the judicious use of antimicrobials it is necessary that both over diagnosis and under diagnosis of infections be avoided. This can be achieved only by adopting appropriate diagnostic stewardship strategy for diagnosing various infections. Strategies for diagnostic stewardship are essential not only for bacterial infections but also for parasitic, viral and fungal infections. In the present study to achieve this objective real time analysis of colonic biopsy tissue was done for invasive amoebiasis by demonstrating motile *E. histolytica* trophozoites in saline wet mount preparation of biopsy so that presumptive diagnosis of amoebic colitis is made in real time with a TAT of 15-30 minutes. The clinician could decide whether to rule out Inflammatory Bowel Disease (IBD) and start metronidazole therapy or not immediately after receiving the microscopy report. Otherwise metronidazole would be prescribed empirically until the histopathology reports and amoebic serology reports are available TAT 24-48 hours (sometimes even more time is taken for reporting depending on workload of the laboratories).

Metronidazole is the standard empirical drug for the treatment of suspected cases of invasive amoebiasis irrespective of acute or chronic infection, whether invasive or not. Because of the indiscriminate use of metronidazole for treatment of diarrhoea, metronidazole resistance has been reported in *E. histolytica* from various parts of the world [4]. It is essential that the specimen be transported immediately to the laboratory. Right test at right time for clinical management requires good communication between laboratory and clinicians which is definitely the need of the hour. The TAT of direct visualisation of motile trophozoites in biopsy is just 15 to 30 minutes in comparison to 12-24 hours TAT in case of histopathology of biopsy or ameobic serology also for that matter.

The wet mount preparation has the advantage of being simple, minimal infrastructure requirement, performed immediately and therefore allows immediate preventive, diagnostic, or therapeutic action where a positive result is found. Wet mount also gives a fair idea about the approximate burden of an infection. It easily helps in identifying the characteristic motility of various parasitic trophozoites and bacteria causing infections in humans. The disadvantage of wet mount microscopy is that it dries within few minutes, making it difficult to visualise or identify the live organism. For later consultation or demonstration, a fresh wet mount preparation needs to be made each time to view slides thus consuming the resources and time of the technical staff [5,6]. Various type of techniques are used for wet mount microscopy [Table/Fig-3] [7,8]. The most common wet mount technique is the saline wet mount used mainly for parasitic infections followed by Potassium Hydroxide (KOH) mount used for fungal infections. List of infections that can be easily diagnosed with simple wet mount microscopy is given in [Table/Fig-4] [9-28]. The TAT for these infections can be reduced to 30 minutes from 24-48 hours which is the TAT for culture and molecular methods used in their diagnosis.

Technique	Purpose
Saline wet mount	To determine motility
Hanging drop preparation	To determine motility
Iodine wet mount	To clearly visualise internal structures, nuclei in ova/cysts present in stool sample
10% KOH mount	To detect fungal elements To dissolve background keratin
India ink preparation	To see capsules of organism
Wet mount Dark field examination	To visualise delicate organisms invisible by light microscope.

[Table/Fig-3]: Different wet mount microscopy techniques [7,8].

In comparison to staining, culture and molecular techniques a wet mount preparation procedure is simple [Table/Fig-5] [28]. A drop of saline or simple dye like India ink, methylene blue, lactophenol cotton blue or KOH solution is put on a glass slide. The sample is added and a cover slip is put and the mount is ready to be examined under the light microscope. Whereas direct smear staining methods have different multistep procedures that require technical expertise, smears need to be prepared followed by heat or chemical fixation and one or more than one dye is needed for staining. Several kinds of differential and special stains are required in staining techniques for direct examination of fixed clinical samples like Gram's stain,

Clinical specimen	Suspected disease	Wet mount technique	Findings
Stool	Cholera.	Hanging drop preparation.	Bacilli showing darting motility.
	Intestinal parasitic diseases.	Saline and iodine wet mount Lactophenol cotton blue wet mount Methylene blue-glycerol wet mount.	Ova/cyst/motile trophozoites/larvae of various parasites seen.
Colon biopsy	Amoebic colitis	Saline wet mount.	Motile trophozoites of *E histolytica* seen.
Urine	Urinary Tract Infection (UTI).	Wet mount of urine.	Bacteruria/pyuria/yeast cells.
	Leptospirosis.	Wet mount Dark field examination.	Loosely coiled motile spirochetes seen.
	Urinary parasites.	Saline Wet mount of centrifuged urine.	Trophozoites of *Trichomonas.* Egg of *Enterobius vermicularis.* Larva of *Strongyloides stercoralis.* Ciliate protozoa like *Balantidium coli.* Microfilaria. Egg of *Schistosoma hematobium.*
Vaginal discharge/swab	Vaginal candidiasis.	Saline wet mount.	Pseudohyphae or budding yeast cells seen.
	Trichomoniasis.	Saline wet mount.	Motile trophozoites showing twitching motility.
Genital ulcer secretions	Primary syphilis.	Wet mount Dark field examination.	Tightly coiled motile spirochetes seen.
Skin/nail scrapings; plucked hair	Dermatophytosis.	10% KOH mount.	Delicate hyphae or cluster of spores seen.
	Tinea infection.	10% KOH mount.	Hyphae or cluster of spores seen.
Nasal swab/biopsy	Rhino-orbital Mucormycosis.	10% KOH mount.	Presence broad sparsely septate fungal hyphae seen.
Sputum/Bronchoscopy and Bronchoalveolar Lavage	Pulmonary mucormycosis.	10% KOH mount.	Presence broad sparsely septate fungal hyphae seen.
Wound/ pus discharge/ aspirate	Actinomycotic mycetoma.	Saline wet mount.	Sulphur granules seen.
	Eumycotic mycetoma.	Saline wet mount.	White/ grayish black granules seen.
	Amoebic liver abscess.	Saline wet mount.	Motile Trophozoites of *E histolytica.*
	Cystic disease of liver.	Saline wet mount.	Hooklets, scolex of *Echinococcus granulosus* (dog tapeworm).
Cerebrospinal Fluid (CSF)	Cryptococcal meningitis.	India ink preparation.	Encapsulated round budding yeast cells seen.
	Listeriosis.	Hanging drop preparation.	Bacteria with tumbling motility seen.
	Primary ameobic meningoencephalitis.	Saline wet mount.	Motile amoeboid trophozoites seen.
	Granulomatous amoebic encephalitis.	Saline wet mount.	Both amoebic cyst and trophozoites can be seen.
Corneal scrapings	Amoebic keratitis.	Saline wet mount.	Both amoebic cyst and trophozoites can be seen.
Blood	Relapsing fever (Borrelia).	Wet mount Dark field examination.	Spirochetes with motility.
	Parasites- filariasis.	Direct mount of anticoagulated blood.	Presence of microfilaria.

[Table/Fig-4]: List of infections diagnosed by direct wet mount microscopy with TAT 30 minutes [9-28].

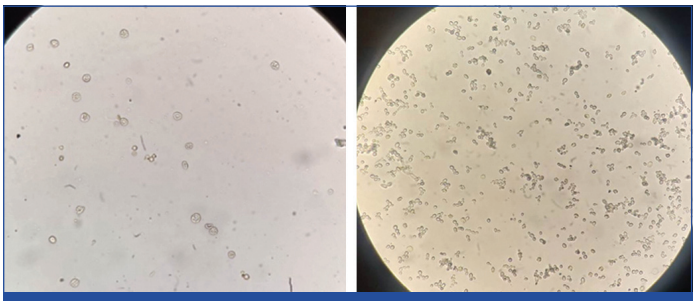
Parameter	Wet mount microscopy	Direct fixed smear staining	Culture	Molecular technique
Procedure	Simple.	Multi step approach, time consuming.	Multi step approach, time consuming.	Multi step approach, time consuming.
Techinical expertise	Easy to perform No expertise needed.	Expertise Required.	Expertise required. Labor intensive.	Expertise Required. Labor intensive.
Turn Around Time (TAT)	30 minutes Strong clinician-laboratory liasion required.	6-24 hours.	24 hours to 6 weeks.	8 to 10 hours.
Specimen transport	Rapid transport needed within 15-30 minutes.	Within 2 hours.	Within 2 hours/transport medium may be needed.	Within 2 hours/Transport medium needed/cold chain need to be maintained for viral infections.
Expense	Cheap and cost effective.	Expensive	Expensive	Expensive
Lab Set-up	Minimum infrastructure required -light microscope only. -glass slide, cover slip. -Saline/KOH/Simple dye.	Complex infrastructure -Light microscope. -Fluorescent microscope. -Phase contrast microscope etc. -Differential stains. -Special stains. -Chemical fixatives. -Bunsen burner or gas for heat fixation. -Various other instruments for preparing biopsy smears.	Complex infrastructure - Bio safety cabinet. - Incubator. - Bunsen burner. - Glassware. - Different culture media reagents and chemicals etc. - Automated instruments.	Complex infrastructure -Different room spaces for pre PCR/ PCR/Post PCR processing. - Equipments like PCR workstation, centrifuges, incubators, vortex etc., - PCR machines. - Nucleic acid extractors. - expensive reagents and kits.
Scope	Limited. Good for positive or high organism load sample.	Wide	Wide	Wide

[Table/Fig-5]: Comparison of wet mount microscopy, staining, culture and molecular techniques [28].

Albert stain, Acid fast stain, Giemsa, fluorescent stains etc., [29]. Moreover, more complex and expensive microscopes like fluorescent microscope, phase contrast microscope etc., are required to view the specially stained slides. Culture and Polymerase Chain Reaction (PCR) techniques also require a complex infrastructure, technical expertise and are time consuming and labor intensive although they have a wider scope and higher sensitivity and specificity. Similarly, for other infections also, mainly bacterial, fungal and parasitic, a wet mount microscopy will be useful in greatly decreasing the TAT for presumptive diagnosis of those infections and thus guide the empirical therapy to be given. Viral infections can also be diagnosed by direct microscopy but it requires electron microscope and complex infrastructure which is beyond the scope of this discussion.

Diagnostic Significance of Wet Mount Microscopy

Bacterial infections: Many clinicians rely on routine microscopic examination of urine without culture as indicator of probable UTI and guide their treatment based on the positive results [Table/Fig-6,7]. Urine culture is the Holy Grail in diagnosis of Urinary Tract Infection (UTI) however the significance of preliminary urinalysis by wet mount microscopy cannot be neglected [30]. Studies have shown that the sensitivity of urine wet mount ranges between 40-70% and specificity between 85-95% in predicting UTI [31,32]. Evaluation of suspected UTI includes history, physical examination and laboratory investigations. Urine wet mount analysis for presence of pus cells (pyuira) and bacteria (bacteruria) are important in the adequate management of UTI [31]. In the absence of significant bacteriuria, presence of pyuria in a symptomatic patient (e.g., acute urethral syndrome) is an indication for treatment and hence the importance of wet mounts microscopy [32].



[Table/Fig-6]: Urine wet mount showing pus cells (400X) suggestive of pyuria.
[Table/Fig-7]: Urine wet mount showing pus cells (400X) suggestive of pyuria.
(Images from left to right)

Vaginal fluid wet mount examination helps to assess the status of vaginal lactobacillary flora, which is an indicator of vaginal infection and pregnancy complications [33]. Fatima A et al., in their study found sensitivity of 85.85%, specificity of 72.89%, positive predictive vale of 64.88% and negative predictive value of 89.55% for urine wet mount in detection of UTI [5]. Leptospirosis is a wide spread zoonotic disease in the world. In India, several outbreaks have been reported especially South-India, mainly between June and October due to heavy rains and floods [34]. Dark field microscopy is used to detect *Leptospires* in body fluids like urine. Thin, bright, actively motile spiral bacilli with characteristic rapid spinning and jerking motility are suggestive of *Leptospires*. The disadvantage of this technique is that it is less sensitive and specific as atleast 10 leptospires/mL must be present for one cell per field to be visible by darkfield microscopy [34].

Listeria monocytogenes is a gram-positive bacterium that is not very common but important cause of severe CNS infections. *L. monocytogenes* infection can lead to meningitis, supratentorial abscesses (commonly in immunocompromised individuals) and even brainstem encephalitis (rhomboencephalitis) in immunocompetent patients. Due to the high mortality and common serious sequelae in survivors, an accurate initial diagnostic approach is important for applying appropriate treatment early [35]. Though early diagnosis is challenging but *Listeria* demonstrates "tumbling motility" in wet mounts of Cerebrospinal Fluid (CSF) and if positive can contribute towards timely patient management.

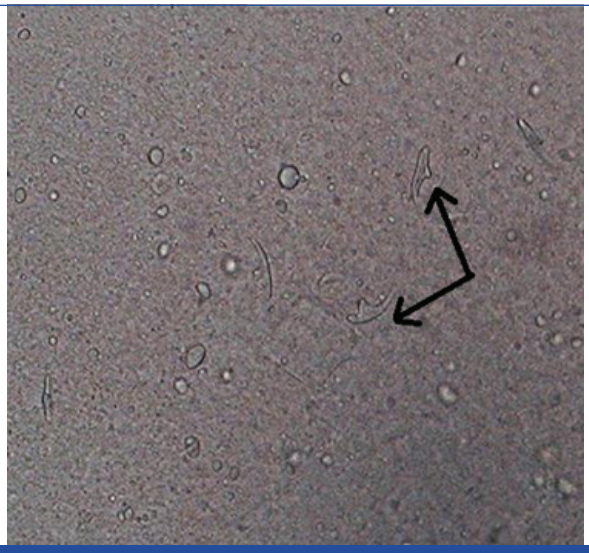
Parasitic infections: Through wet mount microscopy of centrifuged urine sediment, a wide morphological spectrum of parasites causing pyuria and haematuria may be diagnosed. The morphological awareness guides in prompt and effective management in most cases [36]. Parasites commonly found in urine are *Trichomonas, Schistosoma hematobium* and micofilaria [9,37]. *Schistosoma* infection (Bilharziasis) and filarial infection is not common in India [10]. *Trichomonas vaginalis* causing vaginitis and urethritis in females can be seen in urine and vaginal discharge or swab wet mount examination. Both spun urine (centrifuged deposits) in conjunction with vaginal fluid examination improves the detection rate of Trichomonads [11]. Trichomonads also infect the male urogenital tract and are an important sexually transmitted infection. The trophozoites of *T. vaginalis* can be seen in urinary sediments from males as well [12]. Rare reports of urinary balantidiasis have been been seen mostly in immunocompromised patients [13,14]. The trophozoites of ciliated parasite *Balantidium coli* can be seen in urine sediment wet mount microscopy. Even the larvae of *Strongyloides stercoralis* and planoconvex egg of *Enterobius vermicularis* have been reported in urine wet mount [36]. The urine sediment wet mount examination Tetteh-Quarcoo PB et al., examined urine sample in one study microscopically for the presence of *S. haematobium* eggs and 18.0% urine samples were positive [15].

Parasites causing diarrhoea are present commonly in water supplies, infecting a large proportion of the human population in the developing countries [16]. Stool is the most common specimen collected and examined for demonstration of parasites of the gastrointestinal tract. Usually two preparations, saline and iodine wet mount of stool is made on a single slide. The saline wet mount is made up of physiological saline and is an unstained preparation. The advantage of saline wet mount is that it demonstrates the motility of trophozoites. However, internal structures are often poorly visible in saline mount and to overcome this simple stain solutions like iodine, methylene blue, lactophenol cotton blue have been used for preparation of temporarily stained wet mounts of stool sample. Commonly iodine wet mount is prepared from stool sample however many studies have used other stains also for better results. A combination of methylene blue and glycerol in wet mount preparation of fresh stool samples was used by Khanna V et al., for the demonstration of medically important intestinal parasites [16]. They found that methylene blue-glycerol combination provided an excellent contrast between the parasitic structures and the artefacts as compared to saline and iodine mount. A similar type of study was done by Parija SC and Prabhakar PK with similar results with different parasites where they evaluated the use of lactophenol cotton blue wet mount preparation as an alternative to routine saline and iodine wet mounts [17].

Naegleria fowleri, *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Sappinia* sp. are pathogenic free-living amoebae. Chronic granulomatous encephalitis is caused by *Acanthamoeba* spp. and B. *mandrillaris*. *Acanthamoeba* spp. also skin and eye infection. Amoebic Keratitis is infection of the cornea that is associated with contact lens use or corneal trauma and can lead to loss of sight. *Sappinia pedata* has been identified as the cause of a nonlethal case of amoebic encephalitis [18]. The direct detection of the causative agent in corneal scraping specimen is the only reliable diagnostic method for Amoebic Keratitis [19]. Although culture and PCR techniques are definitely more sensitive, however, amoeba density is high in severe infection and the amoebic trophozoites can already be detected by direct microscopy of the clinical sample [19]. Direct wet mount microscopy of CSF sediments after gentle centrifugation of samples is recommended to look for the trophozoites for the diagnosis of *Acanthamoeba* in CSF [20]. Primary Amoebic Meningoencephalitis (PAM) a rare condition, caused by free-living amoeba *Naegleria fowleri*, is associated with fresh water exposure (swimming in ponds

and lakes in the summer). The ameobae penetrate through the nasal mucosa and rapidly progressive meningoencephalitis ensues after a brief incubation period. The clinical course of *N. fowleri* infection is fulminant rapidly progressing to death in a median of five days. Wet mount examination of the CSF reveals motile trophozoites [21]. Presumptive diagnosis of *N. fowleri* infection can be done via direct microscopic examination of the CSF immediately after sample collection although confirmatory diagnosis requires specialised experience, immunohistochemical staining, or Polymerase Chain Reaction (PCR) testing [22]. In a study done by Capewell LG et al., PAM was diagnosed before death (or in the case of the survivors, discharge) in 27% (39 of 142) of the patients [23]. In nearly all (38 of 39) cases, motile amoebas were seen in a wet mount of the CSF.

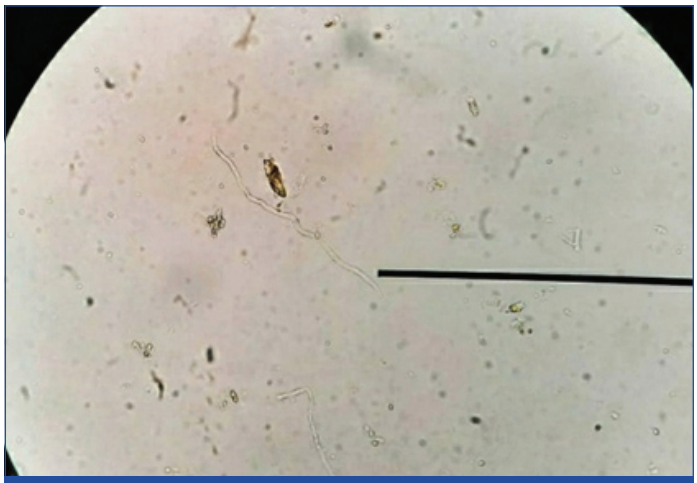
The hooklets and scolex of *Echinococcus granulosus* (dog tapeworm) are visible in the cystic fluid wet mount examination of aspirate from liver in case of hydatid liver disease [Table/Fig-8].



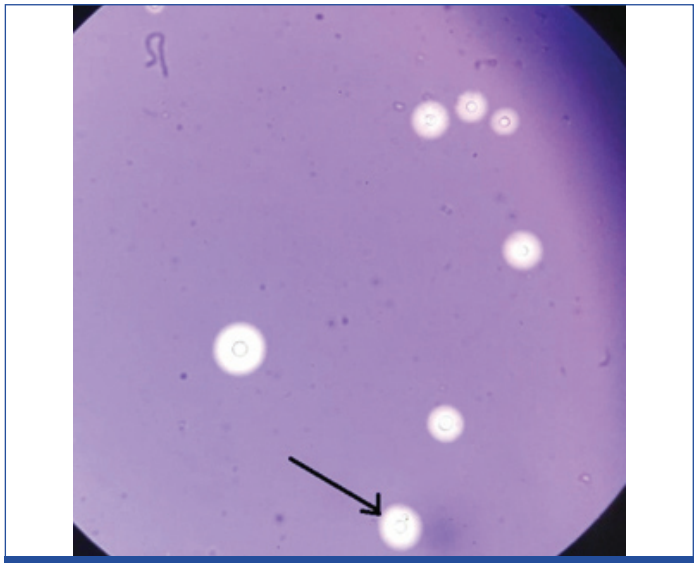
[Table/Fig-8]: Hooklets seen in direct saline wet mount of cystic fluid (400X) from liver suggestive of Hydatid cyst.

Fungal infections: Culture is the gold standard for diagnosing fungal infections but they are expensive and time consuming (requires six weeks incubation). While a 10% KOH mount of clinical samples is clinically useful for early diagnosis of common superficial fungal infections. The KOH is a cheap and readily available. It digests, and clears cellular and keratin debris but leaves fungal hyphae cell wall intact which is resistant to digestion by KOH, thus clearing the background and allows visualisation of fungal elements under a microscope. Gautam M and Bhatia S in their study showed that KOH has higher sensitivity as compared to fungal culture (70 to 100%) for cutaneous dermatophyte infections, Tinea capitis or tinea barbae, Onychomycosis, Tinea versicolor and candidiasis [6]. The KOH mount examination of sputum samples help to identify Aspergillus infection by the presence of V-shaped branching and septate hyphae. In case of Mucormycosis, aseptate hyphae will be seen on KOH mount of clinical sample [Table/Fig-9]. Mucormycosis is a life-threatening fungal infection that occurs in immunocompromised patients. During Coronavirus Disease-2019 (COVID-19) pandemic increasing number of mucormycosis cases has been reported from India [24]. Rapid diagnosis of mucormycosis is important to guide timely initiation of amphotericin B and possible surgical intervention in order to reduce morbidity. The clinical presentation, signs, and radiographic manifestations of mucormycosis are nonspecific and so direct KOH examination of clinical samples like tissue, respiratory secretions, bronchoalveolar lavage, and other body fluids is frequently done [25].

The incidence of cryptococcosis in India is on the rise thus becoming a serious threat. High morbidity and mortality are associated with cryptococcal meningitis and therefore early and timely diagnosis is essential to prevent serious complications [26]. The CSF India ink examination has provides an immediate, specific diagnosis of cryptococcal meningitis within minutes of receiving the CSF sample in the laboratory [Table/Fig-10]. In one study by Saha DC et al., sensitivity and specificity of India ink examination of CSF for *cryptococcus* was found to is 83.3% and 96.49%, respectively [27]. The laboratory diagnosis of cryptococcal meningitis includes direct visualisation of cryptococci via microscopy, culture of the organism and/or the detection of cryptococcal antigens in CSF [38]. Although India ink has low sensitivity and specificity yet it is still widely used for the detection of cryptococci in CSF, particularly in resource-limited countries as it is cheap, rapid and reliable [38,39].



[Table/Fig-9]: KOH mount of nasal biopsy showing broad aseptate hyphae (400X) suggestive of Mucormycosis.



[Table/Fig-10]: India ink preparation of CSF showing round capsulated budding yeast cell suggestive of *Cryptococcus neoformans*.

Limitation(s)

The limitation of this study was that only amoebic colitis cases have been studied here by direct microscopy and rest all infection list has been collected and compiled according to the literature search. Further research needs to be done by correlating the effect of reduced TAT of diagnosis by direct microscopic examination with patient outcome and management not only in amoebic colitis cases but also other infections.

CONCLUSION(S)

Wet mount microscopy should not just remain a teaching tool for training microbiology residents but the significance of this simple although limited in scope diagnostic test for several infectious diseases needs to be reassessed and analysed probably in countries like India with limited resources. In resource limited settings with absence of culture and PCR facilities, a rapid, simple and cost-effective technique like microscopy of wet mount preparation of clinical specimens with a TAT of 15 to 30 minutes is the first step

towards diagnostic stewardship initiative to avoid empirical use of antimicrobial agents atleast in positive or heavily infected cases where organism load is high and therefore presumptive diagnosis can easily be made on wet mount microscopy. Similar studies on larger sample size with direct impact on use of antimicrobial drugs and clinical management is the way forward.

REFERENCES

[1] Savoldi A, Gentilotti E, De Nardo P, Razzaboni E, Bovo C, Carrara E. Ushering in diagnostic stewardship: A step towards antibiotic stewardship. Curr Treat Options Infect Dis. 2020;12:202-14. https://doi.org/10.1007/s40506-020-00224-7.

[2] Morjaria S, Chapin KC. Who to test, when, and for what: Why diagnostic stewardship in infectious diseases matters. The journal of Molecular Diagnostics. 2020;22(9):1109-13.

[3] Patel R, Fang FC. Diagnostic stewardship: Opportunity for a laboratory–infectious diseases partnership. Clinical Infectious Diseases. 2018;67(5):799-801. https://doi.org/10.1093/cid/ciy077.

[4] Petri, William A. Therapy of intestinal protozoa. Trends in Parasitology. 2003;19(11):523-26 .

[5] Fatima A, Jan A, Akhter N, Fomda BA, Suhail M Ahmad J, et al. Evaluation of microscopic screening methods for detection of urinary tract infection. Int J Cur Res Rev. 2017;9(6):44-49.

[6] Gautam M, Bhatia S. Mount the menace! - Potassium hydroxide in superficial fungal infections. Indian J Paediatr Dermatol. 2020;21:343-46.

[7] Zaman RF, Khanum H, Nargis S, Das PK. Comparison of saline, iodine and KOH wet mount preparations for occurrence of parasites in stool samples from patients attending ICDDR. B Bangladesh J Zool. 2017;45(2):159-70.

[8] Washington JA. Principles of Diagnosis. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 10. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8014/.

[9] Mc Pherson RA, Threatte GA, Pincus MR Basic examinationof urine. In: Mc Pherson RA, Pincus MR (eds) Henry's clinical diagnosis and management by laboratory methods, 22nd edn. Elsevier Saunders, New Delhi, 2011; 445-479.

[10] Kali A. Schistosome infections: An Indian perspective. J Clin Diagn Res. 2015. https://doi.org/10.7860/jcdr/2015/10512.5521.

[11] Blake DR, Duggan A, Jaffe A. Use of spun urine to enhance detection of Trichomonas vaginalis in adolescent women. Arch Pediatr Adolesc Med. 1999;153:1222-25.

[12] Kaydos-Daniels SC, Miller WC, Hoffman I, Price MA, Martinson F, Chilongozi D, et al. The use of specimens from various genitourinary sites in men, to detect Trichomonas vaginalis infection. J Infect Dis. 2004;189(10):1926-31.

[13] Bandyopadhyay A, Majumder K, Goswami BK. Balantidium coli in urine sediment: Report of a rare case presenting with hematuria. J Parasit Dis. 2013;37:283-85.

[14] Karuna T, Khadanga S. A rare case of urinary balantidiasis in elderly renal failure patient. Trop Parasitol. 2014;4:47-49.

[15] Tetteh-Quarcoo PB, Akuetteh BK, Owusu IA, Quayson SE, Attah SK, Armah R, et al. Cytological and wet mount microscopic observations made in urine of schistosoma haematobium-infected children: hint of the implication in bladder cancer. Canadian Journal of Infectious Diseases and Medical Microbiology. 2019;2019:7912186. 8 pages. https://doi.org/10.1155/2019/7912186.

[16] Khanna V, Tilak K, Rasheed S, Mukhopadhyay C. Identification and preservation of intestinal parasites using methylene blue-glycerol mount: A new approach to stool microscopy. Journal of Parasitology Research. 2014;2014:672018. 4 pages. https://doi.org/10.1155/2014/672018.

[17] Parija SC, Prabhakar PK. Evaluation of lacto-phenol cotton blue for wet mount preparation of feces. Journal of Clinical Microbiology. 1995;33(4):1019-21.

[18] Da Rocha-Azevedo B, Tanowitz HB, Marciano-Cabral F. Diagnosis of infections caused by pathogenic free-living amoebae. Interdiscip Perspect Infect Dis. 2009;2009:251406. Doi: 10.1155/2009/251406. Epub 2009 Aug 2. PMID: 19657454; PMCID: PMC2719787.

[19] Lorenzo-Morales J, Khan NA, Walochnik J. An update on Acanthamoeba keratitis: diagnosis, pathogenesis and treatment. Parasite. 2015;22:10.

[20] Marciano-Cabral, F, Cabral G. Acanthamoeba spp. as agents of disease in humans. Clin Microbiol Rev. 2003;16:273-307.

[21] Jurado R, Walker HK. Cerebrospinal Fluid. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Boston: Butterworths; 1990. Chapter 74. Available from: https://www.ncbi.nlm.nih.gov/books/NBK398/.

[22] Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri, and Sappinia diploidea. FEMS Immunol Med Microbiol. 2007;50:01-26.

[23] Capewell LG, Harris AM, Yoder JS, Cope JR, Eddy BA, Roy SL, et al. Diagnosis, clinical course, and treatment of primary amoebic meningoencephalitis in the United States, 1937-2013. J Pediatric Infect Dis Soc. 2015;4(4):e68-75. Doi: 10.1093/jpids/piu103. Epub 2014 Oct 23. PMID: 26582886.

[24] Satish D, Joy D, Ross A, Balasubramanya. Mucormycosis co-infection associated with global COVID-19: A case series from India. Int J Otorhinolaryngol Head Neck Surg. 2021;7:815-20.

[25] Walsh TJ, Gamaletsou MN, McGinnis MR, Hayden RT, Kontoyiannis DP. Early clinical and laboratory diagnosis of invasive pulmonary, extrapulmonary, and disseminated mucormycosis (Zygomycosis). Clinical Infectious Diseases. 2012;54(suppl_1):S55-60. https://doi.org/10.1093/cid/cir868.

[26] Saha DC, Xess I, Biswas A, Bhowmik DM, Padma MV. Detection of Cryptococcus by conventional, serological and molecular methods. J Med Microbiol. 2009;58:1098-105.

[27] Saha DC, Xess I, Jain N. Evaluation of conventional & serological methods for rapid diagnosis of cryptococcosis. Indian J Med Res. 2008;127(5):483-88. PMID: 18653913.

[28] Koneman EW. (1997). Color atlas and textbook of diagnostic microbiology (5th ed.). Philadelphia: Lippincott.

[29] Sastry S, Bhat S. Essentials of Medical Microbiology, Chp 3.3: Laboratory diagnosis of Bacterial infections (3rd edition). 2021; Pg 25-50. Jaypee Brothers Medical Publishers, New Delhi.

[30] Bharara T, Sharma A, Gur R, Duggal SD, Jena PP, Kumar A. Predictive role of proteinuria in urinary tract infection. Journal of Clinical and Diagnostic Research. 2017;11(10):DC01-03. https://doi.org/10.7860/JCDR/2017/29615.10720.

[31] Ninama AB, Shah PD. Comparison of various screening methods for presumptive diagnosis of significant bacteriuria. Int J Med Sci Public Health. 2016;5(6):1066-69.

[32] Neelam T, Kavya M, Rungmei M, Nandita D, Meera S. Evaluation of three screening methods for detection of urinary tract infection in antenatal women. J Obstet Gynecol Ind. 2004;54(3):267-70.

[33] Donders Gilbert GG, Vereecken A, Dekeersmaecker A, Bulck BV, Spitz B. Wet mount microscopy reflects functional vaginal lactobacillary flora better than Gram stain. J Clin Pathol. 2000;53:308-13.

[34] Budihal SV, Perwez K. Leptospirosis diagnosis: Competancy of various laboratory tests. J Clin Diagn Res. 2014;8(1):199-202.

[35] Yao M, Zhou J, Zhu Y, Zhang Y, Lv X, Sun R, et al. Detection of Listeria monocytogenes in CSF from three patients with Meningoencephalitis by next-generation sequencing. J Clin Neurol. 2016;12(4):446-51.

[36] Khurana U, Majumdar K, Kapoor N, Joshi D, Goel G, Sharma T, et al. Spectrum of parasitic infections in centrifuged urine sediments from a newly developed tertiary care centre in Central India. J Parasit Dis. 2018;42(4):608-15. https://doi.org/10.1007/s12639-018-1043-6.

[37] Cheesebrough M (2009) District laboratory practice in tropical countries, 2nd edn. Cambridge University Press, New York.

[38] Chisale MR, Salema D, Sinyiza F, Mkwaila J, Kamudumuli P, Lee HY. A comparative evaluation of three methods for the rapid diagnosis of cryptococcal meningitis (CM) among HIV-infected patients in Northern Malawi. Malawi Med J. 2020;32(1):03-07.

[39] Coovadia Y, Mahomed S, Dorasamy A, Chang C. A comparative evaluation of the Gram stain and India ink stain for the rapid diagnosis of cryptococcal meningitis in HIV infected patients in Durban. South African J Infect Dis. 2015;30(2):61-63.

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Microbiology, GIPMER, New Delhi, India.
2. Director Professor, Department of Microbiology, GIPMER, New Delhi, India.
3. Consultant Microbiologist, Department of Microbiology, GIPMER, New Delhi, India.
4. Senior Resident, Department of Microbiology, GIPMER, New Delhi, India.
5. Senior Resident, Department of Microbiology, GIPMER, New Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Abha Sharma,
AC 61, Second Floor, Tagore Garden, New Delhi-110027, India.
E-mail: abha_sh79@rediffmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Jun 24, 2021
- Manual Googling: Jan 17, 2022
- iThenticate Software: Feb 03, 2022 (12%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jun 23, 2021**
Date of Peer Review: **Oct 20, 2021**
Date of Acceptance: **Feb 19, 2022**
Date of Publishing: **Mar 01, 2022**