PREVALENCE AND PROFILE OF BACTERIAL, FUNGAL AND PARASITIC OPPORTUNISTIC INFECTIONS IN PEOPLE LIVING WITH HIV/AIDS

Dissertation submitted for

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DEGREE EXAMINATION

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

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CERTIFICATE

This is to certify that this dissertation work entitled **"PREVALENCE AND PROFILE OF BACTERIAL, FUNGAL AND PARASITIC OPPORTUNISTIC INFECTIONS IN PEOPLE LIVING WITH HIV/AIDS"** is a bonafide work done by **DR. C. JUSTINE AUXILIA IRENE**, Postgraduate student, Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-600003, under our direct supervision and guidance.

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DECLARATION

I declare that the dissertation entitled **"PREVALENCE AND PROFILE OF BACTERIAL, FUNGAL AND PARASITIC OPPORTUNISTIC INFECTIONS IN PEOPLE LIVING WITH HIV/AIDS"** is submitted by me for the degree of M.D. Microbiology, is the record work carried out by me during the period of July 2016 to June 2017 under the guidance of **Prof.Dr.U.UMADEVI, M.D.,** Professor, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to The Tamil Nadu Dr.M.G.R. Medical University, Chennai, in partial fulfilment of the University regulations for the award of the degree of M.D., Microbiology (Branch IV) examination to be held in May 2018.

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CERTIFICATE – II

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Introduction

INTRODUCTION

Human Immunodeficiency Virus is essentially an infection affecting the immune system. The main clinical manifestation is due to progressive and profound defect in cell mediated immunity leading to infection by variety of normally innocuous agents which become the major source of morbidity and mortality[1]. HIV presently accounts for the highest number of deaths caused by any single infectious agent. The threat to their life being not from virus alone but due to opportunistic infections (OIs) and associated complications[2].

According to the UNAIDS, approximately 36.7million people were living with HIV/AIDS (PLHA) in 2016 globally with adult population being affected predominantly accounting to approximately 34.5million(94%) and 1million death attributed to AIDS related illness. UNAIDS 2016 statistics of India revealed 2.1million people were living with HIV with adult population accounting to 2million (95%) and 62,000 death due to AIDS related illness[3,4] .

The hallmark of HIV disease is a profound immunodeficiency resulting primarily from a progressive quantitative and qualitative deficiency of the subset of T Iymphocytes referred to as helper T cells(CD4)[5]. Patients with CD4+ T cell levels below certain thresholds are at high risk of developing a variety of opportunistic diseases particularly the infections and neoplasms that are AIDS-defining illnesses.

The organisms causing OIs are usually responsible for asymptomatic or mildly symptomatic, self-limiting infections in immunocompetent individuals whereas in HIV infected patients, these infections may be severe or even life threatening.

Opportunistic infections had been the major cause of death in people living with AIDS. Since the advent of HAART, the incidence of OIs has markedly reduced thereby increasing the survival[6]. Nevertheless significant AIDS associated OIs still persists because of variability in accessing ART owing to:-

(i) Unawareness of HIV infection, hence present with OI as initial indicator of the disease. The first clue towards the HIV pandemic were 5 homosexual males who presented with opportunistic infection without any previously known immunodeficiency in USA[1,7]. Even in recent days, patients present directly with opportunistic infection which is the only clue towards the diagnosis of HIV infection.

(ii) Some patients though aware of their HIV status, do not take ART due to psychosocial or economic factors and (iii) some patients enrolled for ART, do not attain adequate virologic and immunologic response due to inconsistent retention in care, poor adherence, unfavourable pharmacokinetics or unexplained biologic factors[6]. Hence the knowledge about the opportunistic infection prevalent in a region among HIV infected individuals is essential to aid in the diagnosis and treatment of HIV.

The incidence of OIs depends on the level of immunosuppression and on the endemicity of the pathogen[2]. Once the CD4 count falls below 200cell/µl, the cumulative risk of developing OIs is 33% by 1 year and 58% by 2 years. Thus the CD4 count serves as the crucial parameter for monitoring people living with HIV/AIDS as it predicts the incidence of OIs and mortality[8]. In view of the same, initiation of primary prophylaxis for opportunistic infections is based chiefly on CD4 count(<200cells/µl)[9]. Cure for HIV/AIDS may not be achieved but most of the opportunistic infections can be prevented or effectively treated, which will not only prolong the life expectancy of an HIV infected individual but also decrease the morbidity.

The various opportunistic infections seen in HIV infected patients include *Mycobacterium tuberculosis* infection, Disseminated *Mycobacterium avium* complex, recurrent bacterial pneumonia, mucocutaneous candidiasis, Cryptococcosis, *Pneumocystis jirovecii* pneumonia(PCP), Histoplamosis, Coccidioidomycosis, Penicilliosis, *Toxoplasma gondii* encephalitis, Cryptosporidiosis, Microsporidiosis, Varicella-zoster virus infection, Human Papilloma virus infection etc. Tuberculosis is the most common opportunistic infection prevalent in India followed by Candidiasis, Cryptosporidiasis, Herpes Zoster, *Pneumocytitis jirovecii* pneumonia, Bacterial pneumonia and Cryptococcal meningitis[6].

The treating physician must have a knowledge about the prevalent OIs in that geographical region, their diagnosis, prevention and treatment to provide comprehensive, quality care to the patients. The profile of pathogens responsible for OIs varies from country to country and even from region to region within the same country and with the level of immunosuppression[10]. Considering this fact, this study was conducted to determine the prevalence of bacterial, fungal and parasitic opportunistic infections in HIV seropositive patients and its correlation with the CD4 count levels. Hence, the common pathogens causing OIs in PLHA with special reference to those included in the list of AIDS defining illness as per the CDC criteria was investigated and correlated with CD4 count.

Aims and objectives

AIMS & OBJECTIVES

AIM:

- To study the prevalence of bacterial, fungal and parasitic opportunistic infection in People living with HIV/AIDS (PLHA).

OBJECTIVES

- To determine the prevalence and the profile of bacterial , fungal and parasitic opportunistic infections in People living with HIV/AIDS by processing the various clinical specimens with standard microbiological methods.
- To determine the Antimicrobial susceptibility pattern of the bacterial and fungal isolates obtained.
- To correlate the CD4 count with the spectrum of opportunistic infections.

Review of literature

REVIEW OF LITERATURE

HISTORICAL REVIEW:

The initial clue towards HIV epidemic was in June 1981, when five homosexual men presented with *Pneumocystis jirovecii* pneumonia in the absence of any previously known immunosuppression were described by the Centers for Disease Control and Prevention(CDC) in Morbidity and Mortality Weekly Report[1,7]. Since the epidemic was first recognized among homosexual men, the acronym GRID (Gay-related immune deficiency) was proposed initially. Later it was found that the disease affects intravenous drug users and blood transfusion recipients, hence the term AIDS(Acquired Immunodeficiency Syndrome) was adopted. In 1983, Montagnier from France and Gallo from USA individually isolated the retrovirus responsible for the illness and named it Lymphadenopathy-associated virus(LAV) and Human T-cell leukemia virus type III (HTLV-III) respectively[1] . Subsequently, the International Committee for the Taxonomy of Viruses placed it in the family Retrovirus, Genus Lentivirus and was named the Human Immunodeficiency Virus(HIV). By April 1985, a test to detect antibody from blood was licensed. In 1986, HIV-2 which was related but immunologically distinct human retrovirus was found. Zidovudine was first used for AIDS patients in the year 1987. In December 1, 1988 the First World AIDS day was held. HAART regime was instituted from the year 1995[1].

In 1989, the Guidelines for the Prophylaxis against PCP for HIV-infected patients became the first HIV-related treatment guideline published by U.S Public Health Service. Later in 1995, the guidelines was expanded to include the prophylaxis for all the HIV-related opportunistic infections and Infectious Disease Society of America joined as co-sponsor[6].

In 1986, Dr. Suniti Solomon diagnosed the first patient infected with HIV in India at the Govt. General Hospital(now RGGGH), Chennai among female sex workers from Mumbai. In 1987, the Government of India established the National AIDS Control Program to restrain the spread of HIV and promote national efforts against HIV/AIDS. Government of India started the free Antiretroviral Therapy(ART) programme on April 1st 2004[11].

In 2007 at Berlin, Stem Cell transplant for a HIV positive patient was done as a treatment for Leukemia from a donor missing one of HIV's key co-receptor CCR5 and tested negative for HIV thereafter. This has led the researchers in finding a complete cure to HIV though not successful so far[12].

EPIDEMIOLOGY:

HIV Epidemiology: According to the UNAIDS, approximately 36.7million people were living with HIV/AIDS(PLHA) in 2016 globally with adult population being affected predominantly accounting to approximately 34.5million(94%) and 1million death attributed to AIDS related illness. UNAIDS 2016 statistics of India

revealed 2.1million people were living with HIV with adult population accounting to 2 million (95%) and 62,000 death due to AIDS related illness[3,4] .

Epidemiology of Opportunistic infections[6]

Tuberculosis

The estimated annual risk of reactivation with TB disease among those with untreated HIV infection and Latent Tuberculosis Infection (LTBI) is 3% to 16%. In recent years there have been fewer than 1000 new cases of HIV/TB co-infection identified per year in the United States.

Disseminated *Mycobacterium avium* complex disease

M. avium is the etiologic agent in >95% of patients with AIDS who acquire disseminated MAC disease. An estimated 7% to 12% of adults have been previously infected with MAC, although rates of disease vary in different geographic locations.

Bacterial pneumonia

According to the UNAIDS report, the estimated rate of pneumococcal pneumonia in patients with AIDS (1,094 cases per 100,000) was 55 times higher than in HIV-uninfected individuals (20 cases per 100,000).

Mucocutaneous candidiasis

Oropharyngeal and esophageal candidiasis are common in HIV-infected patients. Most such infections are caused by *Candida albicans*.

Pneumocystis jirovecii pneumonia

Before the widespread use of PCP prophylaxis and antiretroviral therapy (ART), PCP occurred in 70% to 80% of patients with AIDS; the course of treated PCP was associated with a 20% to 40% mortality rate in individuals with profound immunosuppression. Approximately 90% of PCP cases occurred in patients with CD4 T-lymphocyte (CD4 cell) counts <200 cells/mm^3.

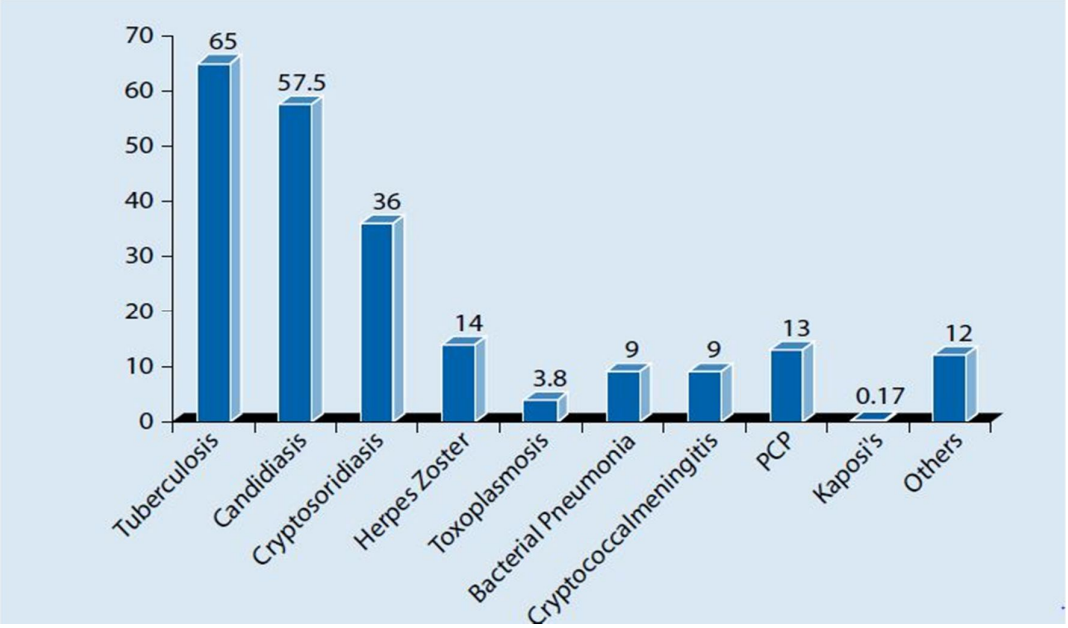
Cryptosporidiosis

Cryptosporidiosis remains a common cause of chronic diarrhea in AIDS patients in developing countries, with up to 74% of diarrheal stools demonstrating the organism. In developed countries, cryptosporidiosis has decreased and occurs at an incidence of <1 case per 1000 person-years in patients with AIDS.

Herpes Zoster infections

The incidence of herpes zoster is >15-fold higher for HIV-infected adults than for age-matched controls. Herpes zoster can occur in HIV-infected adults at any CD4 T lymphocyte (CD4) cell count, but frequency of disease is highest with CD4 counts of <200 cells/μL.

Figure 1: Opportunistic infections among patients with AIDS in India[2].



MOLECULAR EPIDEMIOLOGY:

There are two main types of HIV causing disease in humans: HIV-1 and HIV-2

HIV-1 has four phylogenetically distinct groups, termed group M(main), group O(outlier) and group N(non-M, non-O) and group P. Group M, O and N evolved from Chimpanzee simian immunodeficiency virus(SIVcpz) whereas group P from gorilla SIV (SIV gor). 95% of HIV infection globally is caused by Group M viruses[12]. Group O infection is common occurring primarily in Central Africa(predominantly in cameroon) while group N is rare and limited to Cameroon. HIV-1 show extreme genetic diversity with atleast nine distinct subtypes (or clades) of Group M having 17-35% genetic sequence is seen. Subtype C is found predominantly in Southern Africa, Ethiopia and India and is responsible for nearly half of HIV infection globally. Subtype A causes 12% HIV

infection globally and has wide geographic distribution while subtype B predominates in America, Western and eastern Europe and Australia[13].

HIV-2 is a distinct retrovirus closely related to HIV-1 and is said to have evolved from sooty mangabey SIV(SIV smm). Though reported worldwide, it is more prevalent in Africa. Patients with HIV-2 infection show lower viral loads, reduced rate of transmission and slower progression to immunodefiency compared to patient with HIV-1 infection[14].

MORPHOLOGY OF HIV[1,13]:

The Human Immunodeficiency Virus which causes Acquired Immunodeficiency Syndrome(AIDS) belong to the Family Retroviridae and Genus Lentivirus.

On Electron Microscopy, the virus appears spherical with an outer lipid bilayer(envelope) and a nucleocapsid with a dense, cone shaped core.

The lipid bilayer is derived from the host cell membrane during budding of the mature infectious virus particle. The outer membrane of the envelope consists of 72 spiked knobs assembled as trimers of the envelope protein gp120 bound to the transmembrane protein gp41. The membrane is rich in Cholesterol and several host proteins, most significantly MHC class II proteins

The nucleocapsid is icosahedral in symmetry with a cone shaped core which is broad at one end and narrow at the other. Each mature virion consists of

two molecules of single stranded RNA surrounded by three proteins encoded by gag gene namely,

1. p17- matrix protein
2. p24-major capsid protein
3. p7-nucleoprotein which is bound tightly to viral RNA.

Along with the nucleic acid and gag proteins, the core contains transfer RNA(tRNA), viral protein R(vpr) and other enzymes like protease, reverse transcriptase and integrase

HIV GENOME[13,15]:

The proviral DNA is 9.7kb in length and has genes which code for structural and regulatory proteins.

HIV genome has three structural genes (coding for the structural proteins) namely gag-pol-env gene from 5' end flanked by two complete viral Long Terminal Repeats(LTR) containing transcriptional regulatory sequences, RNA processing signals, packaging sites, and the integration sites.

1. gag gene- encodes for core and matrix proteins namely p17 and p24,p6, p7 respectively
2. pol gene- encodes for viral Protease(p10), Reverse Transcriptase(p66,p51) and Integrase (p32)
3. env gene- encodes for outer and inner transmembrane protein gp120 and gp41 respectively

There are 6 non-structural or regulatory genes (coding for the accessory proteins or the regulatory proteins) arranged in a complex series of open reading frames

1. vif (Viral Infectivity factor gene)- influences the infectivity of the viral particles by efficient cell-free transmission.
2. vpr (Viral protein R)- enhances viral replication, G_2 /M phase arrest.
3. tat (Trans-activator of transcription)- major viral trans-activator, causes immune suppression.
4. rev (Regulator of expression of virion protein)- enhances RNAs expression
5. vpu (Viral protein U)- Present only in HIV-1, enhances virion release and reduces expression of CD4 and MHC class I on the cell surface.
6. nef (Negative regulatory factor)- regulates viral replication based on strain and cell type, reduces CD4 and MHC class I expression.
7. Vpx (Virion protein X)- Present only in HIV-2, packaged into the virion.

REPLICATION CYCLE OF HIV[13,1]:

Steps in viral replication include attachment, penetration, uncoating, DNA synthesis, nuclear transport, integration, particle assembly and budding.

1. ATTACHMENT:

Replication cycle begins with the binding of V_1 region of gp120 protein on the viral envelope with high affinity to the CD4 molecules expressed predominantly on T-helper cells but also on the surface of monocytes, macrophages, dendritic cells and Langerhans cells. After binding to the CD4 molecule, the gp120 protein undergoes conformational changes which facilitate it

to bind to either of the co-receptors CCR5 or CXCR4 expressed on the host cell. These co-receptors belong to the family of seven-transmembrane-domain G protein-coupled cellular receptors which determine the cellular tropism of the virus.

2. PENETRATION/FUSION:

Due to the conformational changes occurring in gp120 protein during attachment, the now exposed gp41molecule penetrates the target cell plasma membrane bringing the virion and target cell at close proximity resulting in fusion.

3. UNCOATING:

Phosphorylation of viral matrix protein by MAP kinase and pH changes regulated by the nef gene results in uncoating which results in release of viral RNA and viral enzymes in the host cytoplasm.

4. DNA SYNTHESIS:

The viral Reverse transcriptase enzyme mediates the transcription of viral genomic RNA into DNA as the viral complex called the pre-integration complex consisting of gag matrix protein, accessory vpr protein and viral integrase along with the above traverse the cytoplasm to reach the nucleus.

5. NUCLEAR TRANSPORT:

The transport of the pre-intergration complex into the nucleus through the nuclear pore is an energy requiring process mediated by the Nuclear Localisation Signals present on viral gag, vpr and integrase protein.

6. INTEGRATION:

The integration of the viral dsDNA with the host cell chromosome is mediated by the viral enzyme Integrase and thus Provirus is formed.

7. BIOSYNTHESIS:

Production of the viral particles depends on cellular and viral factors required for activation of the viral promoters. Some degree of cellular activation is necessary for transcription of the pro-viral DNA into genomic RNA and mRNA which are transported from the nucleus to cytoplasm where the latter translates to form various viral proteins.

9. PARTICLE ASSEMBLY AND BUDDING:

Viral proteins are assembled from outermost to innermost p17, p24, Protease, Reverse Transcriptase, Integrase. The nucleoprotein helps in encapsidation. Then gp160 cleaved in Endoplasmic Reticulum and Golgi Apparatus into gp120 and gp 41.

Budding occurs in special regions of lipid layer called lipid rafts where the Nucleocapsid acquires the envelope. Protease catalyses the cleavage of gag and pol precursors and yields mature virion.

MODES OF TRANSMISSION[5,8,16]:

1. Sexual transmission (heterosexual, homosexual)
2. Parenteral (transfusion of infected blood and blood products, needle prick)
3. Mother to Child transmission (during pregnancy, delivery and breast feeding)

Sexual transmission :

Most common mode of transmission worldwide. Heterosexual transmission is more common particularly in developing countries though high risk of transmission is seen in male homosexual. 75 to 80% of HIV infection in adults are transmitted through unprotected sexual Intercourse[17]. (Heterosexual – 70% which is common in developing countries, Homosexual –5 to 10% common in North America, Europe, Australia). There is strong association between anal intercourse and HIV as there is only a thin, fragile rectal mucous membrane separating semen from the cells which are the targets for HIV[17]. Infection with microorganisms like *Treponema pallidum, Haemophilus ducreyi, Neisseria gonorrhoea, Chlamydia, HSV, Trichomonas vaginalis* cause genital ulcers and increase the risk of transmission of HIV

Parenteral transmission :

Blood transfusion, blood products, or transplanted tissue with HIV can cause infection in the recipient. It is estimated that >90% people exposed to HIV tainted blood products become infected. Hence screening of blood donors for HIV infection is mandatory.

Not only intravenous puncture but also subcutaneous or intramuscular injections with needle infected with HIV can transmit infection in drug abusers or health care professionals. HIV infection can be transmitted at the rate of 63 per 10,000 exposure in case of needle sharing during injection drug use and 23 per 10,000 exposure in percutaneous needle stick injury. Health Care Workers develop HIV by percutaneous injuries or by contact of infected material with non intact skin or mucous membrane. The risk of transmission of HIV by percutaneous injuries is 0.3% and by mucocutaneous injuries is 0.09%.

Perinatal transmission:

Few factors which increase perinatal transmission are advanced maternal stage, increased viral titres, decreased vitamin A levels, chorioamnionitis, maternal anaemia, etc., When the maternal viral load is less than 1000 copies/ml, the risk of transmission to fetus is extremely unlikely but as the load increases to more than 1 lakh copies/ml, the rate of transmission increases to 40%.

MECHANISM OF IMMUNE DYSFUNCTION[8]

The main mechanism of immune dysfunction in HIV infection is by depletion of CD4 T lymphocytes which is a consequence of furious viral replication rate in these cells. About 10^9 CD4 T lymphocytes are killed and replaced in an HIV infected individual. Three major mechanisms have been implicated in CD4 depletion by HIV: direct virus mediated cytolysis, virus induced apoptosis and indirect killing through immune effector mechanism.

IMMUNE RESPONSE[5,13,18]:

Both Humoral and Cell mediated immunity play an important role in HIV infection.

Humoral Immune Response :

Antibodies appear within 6 to 12 weeks of infection. These are the binding antibodies detected by ELISA & Western Blot assay. The first antibodies detected are those formed against gag gene proteins p17 & p24 followed by antibodies to env gene proteins gp160, gp120, gp41and pol proteins p31, p51 and p66. Most of the neutralizing antibodies are formed against the hypervariable region of gp120 protein which is known as the V3 loop region. Antibodies which mediate Antibody Dependent Cell mediated Cytotoxicity and antibodies directed against gp41 also help infection of cells by HIV and this phenomenon is known as **antibody enhancement**. Certain antibodies kill the uninfected also along with infected cells and this is known as **bystander killing.** The infected cells are killed by Complement also.

Cellular Immune Response :

This is mediated by CD4 Helper T cells and CD8 Cytotoxic T cells. Though CD4 cells are the targets of HIV, they undergo proliferation and secrete IL-2 & IFN-γ. CD8 cells produce perforins and cause destruction of HIV infected cells bearing class I MHC molecules.CD8 cells inhibit the replication of HIV and this is mediated by chemokines MIP- 1α and MIP-1β.

NATURAL HISTORY OF HIV INFECTION[8,14]:

Different phases of HIV infection occurs during a period of 8 and 12 years. The three distinct phases in the HIV infection are

1.Primary HIV infection: Also called as Acute Infection Phase. It is a transient condition accompanied by initial rapid plasma viremia exceeding 10,00,000 RNA copies/ml, massive decrease in blood and tissue CD4 especially gut and lymph nodes and increase in blood CD8 T-cell count. Signs and symptoms appear 2-4weeks after exposure and generally last less than 14days. Diagnosis relies on positive p24 antigen or plasma viral RNA >50,000copies/ml.

2.The chronic asymptomatic phase: It is a long latency phase following primary HIV infection, without any signs or symptoms with stable viral replication and CD4 count. The virus replicates in the gut and lymphoid tissues causing its progressive anatomic and functional deterioration which latter result in rapid increase in viremia and decrease in CD4 T cell count thus transforming into overt AIDS.

3. Overt AIDS(Acquired Immune Deficiency Syndrome): End stage, which when untreated leads to death in 2-3years. The opportunistic infections significantly increases as the CD4 count declines.

Pattern of Progression[5,14]: The progression of the disease may vary from person to person and certain patient groups were identified based on pattern of disease progression.

Typical Progressors: The median time interval to development of AIDS after acquiring infection is 8-10years in the absence of therapy. 80-90% of HIV infected individuals are “typical progressors”

Rapids progressors: They develop AIDS and reach end stage within 2-3years with plasma viral level after primary HIV infection being $>10^5$ copies/ml and rapidly decreasing CD4 count. About 5-10% of PLHA fit this profile[(14)].

Long-term non progressors: A subgroup of slow progressors with documented HIV infection for 8-10years, not on treatment, have no signs of disease progression, high CD4 count constantly and viral load <5000HIV RNA copies/ml of plasma. About 5% of PLHA fall in this category

Elite controllers: Also called as natural controllers, are a subset of LTNPs.They are HIV infected individuals with chronic infection, <50copies/ml(undetectable level) irrespective of the period with control of viremia.

WHO CLINICAL STAGING OF HIV IINFECTION[(2)]:

Clinical Stage I

1. Asymptomatic Infection
2. Persistent Generalised Lymphadenopathy

Clinical Stage II

1. Unintentional Weight loss < 10% of body weight
2. Minor mucocutaneous manifestations (dermatitis, recurrent oral ulcers, angular cheilitis, fungal nail infections)
3. Herpes zoster
4. Recurrent upper respiratory tract infections

Clinical Stage III

1. Unintentional Weight loss > 10% of body weight
2. Chronic diarrhoea > 1 month
3. Fever > 1 month
4. Oral Candidiasis
5. Oral Hairy Leucoplakia
6. Pulmonary TB
7. Severe bacterial infections
8. Vulvo vaginal Candidiasis

Clinical Stage IV

1. HIV wasting syndrome
2. *Pneumocystis jirovecii* pneumonia

3. Toxoplasmosis of brain
4. Cryptosporidiasis with diarrhoea > 1 month
5. Isosporiasis with diarrhoea > 1 month
6. Cryptococcosis (extrapulmonary)
7. Cytomegalovirus infection of an organ other than liver, spleen or lymph node
8. Herpes Simplex infection(mucocutaneous)
9. Progressive Multifocal Leucoencephalopathy
10. Any disseminated endemic mycosis
11. Candidiasis of esophagus, trachea, bronchus, lung
12. Atypical Mycobacterial infection
13. Non typhoid *Salmonella* septicaemia
14. Extrapulmonary TB
15. Lymphoma
16. Kaposi's Sarcoma
17. HIV encephalopathy

AIDS DEFINING ILLNESS[(2)]:

- Significant weight loss (>10% of body weight) within last one month/cachexia (not known to be due to a condition other than HIV infection)
- Chronic diarrhoea (intermittent or continuous) for >1 month or prolonged fever (intermittent or continuous) for >1 month
- Tuberculosis: Extensive pulmonary, disseminated, miliary, extrapulmonary

- Neurological impairment preventing independent daily activities, not known to be due to conditions unrelated to HIV infection (e.g. trauma)
- Candidiasis of the oesophagus (diagnosable by oral candidiasis with odynophagia)
- Clinically diagnosed life-threatening or recurrent episodes of pneumonia, with or without aetiological confirmation
- Kaposi sarcoma
- Cryptococcal meningitis
- Cerebral toxoplasmosis
- CMV retinitis
- *Pencillium marneffei* infection
- Recurrent herpes zoster or multidermatomal herpes infection
- Disseminated *molluscum contagiosum*

LABORATORY DIAGNOSIS OF HIV [5, 14,19,20] :

By early 1985, antibody based tests were developed in USA, by 1996 p24 antigen capture assay was developed and by 2002, nucleic acid testing was developed. The diagnosis of HIV depends on detection of HIV antibodies and direct demonstration of HIV or one of its components. Antibodies to HIV start to appear from 2-12 weeks of infection (30).

Specimen Collection & Transport

Specimen:

- For serological tests(antibody and antigen detection) serum/plasma/whole blood,
- Whole blood collected in K2/K3 ethylene diamine tetra acetic acid(EDTA) is used for CD4 enumeration,
- For DNA/RNA PCR, Dried blood spot(DBS) or whole blood with EDTA is used

The samples must be properly labelled.

Specimen transport:

- 3-layer system is used for transport of the specimen
- The tube containing specimen is placed in a leak proof container(eg. Plastic bag with zip-lock), This container is packer inside a cardboard box with sufficient cotton gauze to absorb blood in case of leak.

Specimen storage:

- Sera: 2-8^{o}C upto 1 week, -20□C for longer duration
- EDTA-blood for CD4 enumeration: ambient temperature upto 48hours. Do not refrigerate
- Plasma for HIV-1 viral Load: Plasma should be separated within 6hours of collecting whole blood and stored at -20□C for longer durations.

Objective of testing:

- Transfusion and transplant safety
- Diagnosis of HIV infection in both asymptomatic and symptomatic individuals
- Prevention of parent to child transmission
- For Post-Exposure Prophylaxis(PEP)
- Epidemiological surveillance using unlinked anonymous HIV testing
- Research

DIAGNOSTIC TESTS[14] :

Tests for Detection of Anti-HIV Antibody:

Screening tests

1. ELISA

2. Rapid tests:

Immunoconcentration/Dot Blot Assay(vertical flow). Immunochromatographic Assay(lateral flow), Agglutination assay, Dipstick and comb assay based on Enzyme Immune Assay(EIA)

Supplemental tests

1. Immunofluorescent assay
2. Western blot
3. Line Immuno assay
4. Radio Immuno Precipitation Assay

Other tests :

1. P24 antigen tests
2. DNA PCR
3. RNA PCR

Alternative tests :

1. Saliva HIV tests
2. Urine HIV tests

SCREENING TESTS[14]: Based on the principle of the assay and type of antigen used, serological tests are classified as first to fourth generation.

Table 1: Generation of Anti-HIV antibody tests

Generation	Antigen/Antibodies	Comment/Characteristic
First	Antigens from HIV lysates	Lack of sensitivity and specificity
Second	Recombinant proteins and/or synthetic peptides	Improved sensitivity
Third	Recombinant proteins and/or synthetic peptides in an antigen sandwich configuration	Very high sensitivity and able to detect IgM antibody in addition to IgG antibody; reduces the window period considerably. Detects HIV-1 and HIV-2 simultaneously
Fourth	Detection of both HIV antigen(p24) and both antibodies, IgG and IgM	Further reducing the window period

ELISA:

This is the widely used sensitive test for HIV infection because of its high sensitivity. The antigen is coated on microtitre wells. The test serum is added. If antibodies are present, it binds to the antigen. After washing, anti human immunoglobulin linked to a suitable enzyme is added followed by a colour forming substrate. If the test serum contains anti HIV antibodies, a colour is formed which can be detected visually. There are four generations of ELISA.

1. First generation – whole viral lysate
2. Second generation – Recombinant antigen
3. Third generation – synthetic peptide
4. Fourth generation – antibody + p24 antigen (HIV duo)

Using first and second generation ELISA, antibodies can be detected in 6-12 weeks. With third generation ELISA, antibodies can be detected in 3 weeks. With fourth generation ELISA, since it detects p24antigen also HIV can be diagnosed in 2 weeks.

RAPID TESTS :

These tests yield results within 30 minutes with sensitivity and specificity comparable to ELISA. The most commonly used rapid anti-HIV tests are based on the principle of Immunoconcentration/Dot Blot Assay(vertical flow). Immunochromatographic Assay(lateral flow), Agglutination assay, Dipstick and comb assay based on Enzyme Immune Assay(EIA)

Advantages:

- Point of care tests
- Don't require special equipment
- Technically simple to perform
- Stores in ambient temperature(20-25□C)

WESTERN BLOT :

This assay is based on the fact that various HIV antigens of different molecular weight induces the production of specific antibodies. The antibodies to each component produces a band. So the HIV proteins are separated according to their molecular weight and electrophoretic mobility by polyacrylamide gel electrophoresis and blotted on strips of nitrocellulose paper. The strips are reacted with test sera and then with anti human immunoglobulin conjugated with enzyme. A suitable substrate is then added which produces a colour band where specific antibody has reacted with the separated viral antigen. The position of the band indicates the antigen with which the antibody has reacted.

Interpretation :

Positive Western Blot Criteria :

1. WHO – 2 env with/without gag/pol
2. CDC – Any two p24, gp41, gp120, gp160

Negative – no bands

Indeterminate – bands present but does not satisfy the criteria.

Limitations of Antibody Assay:

- Antibodies not detected in window period
- Not useful in children below 18months of age

P24 ANTIGEN TEST :

P24 antigen tests are also ELISA based and use antibodies to capture the disrupted antigen. This antigen becomes detectable as early as two weeks and lasts for 3-4 weeks and elevates again during late stage of AIDS. But it is less sensitive.

Uses :

1. During the window period
2. To detect HIV infection in newborn(not reliable)
3. During late stage of HIV/AIDS(immune collapse).
4. Monitoring progress of HIV infection

Viral RNA Detection:

It is the “Gold standard” method for confirmation of HIV infection. Three different techniques are available Reverse transcriptase Polymerase Chain reaction (RT-PCR), Nucleic Acid Sequence Based Amplification(NASBA), branched DNA Assay an Real-time RT-PCR. In this the target HIV RNA is amplified enzymatically invitro by chemical methods. It is extremely sensitive and specific detecting even few copies of viral RNA. HIV nucleic acid is detectable as early as 12 days hence used in diagnosing during window period. Can be used to monitor viral load, typing HIV and detecting drug resistance.

DNA PCR:

It detects proviral DNA. It is extremely useful in detecting paediatric HIV, to differentiate latent HIV infection from active viral transcription, viral load estimation and genotype detection.

NEWER TESTS :

ORASURE- SALIVA HIV TEST :

Non-invasively collected specimens like oral fluid, saliva and oral mucosal transudate are used. These systems detect antibodies comparable to or exceeding serum samples.

URINE TESTS :

IgG antibodies are found in urine. The collection of urine is simple, noninvasive and so more useful in developing countries where trained technicians are not available for collecting blood.

LAB MONITORING OF PATIENTS WITH HIV INFECTION [(14)]:

The laboratory tests used for monitoring the stage and progression of HIV infection are

1. Immunological tests
    - CD4 T cell enumeration
2. Virological assays
    - HIV RNA load assay
    - Measurement of HIV p24
    - Reverse Transcriptase activity assay

CD4 COUNT :

CD4 count measures the degree of immunosuppression. CD4 progressively declines as the immune function decreases. It is used in staging the disease, monitoring disease progression, serves as a guide to start ART, determining treatment failure. CD4 cell count is the best predictor of disease progression and it is cheaper than viral load. So it is useful in developing and poor nations [21].

The WHO 2010 recommendations states that all HIV positive adults with CD4 count less than 350 cells/µl should be started on ART with or without symptoms. WHO defines immunological failure as fall of CD4 count to baseline or below or 50% decrease of CD4 count from on treatment peak value or persistent CD4 count below 100 cells/µl.

The U.S. CDC uses CD4 count and divides AIDS into three categories A, B, C. Category A CD4 count >500 cells/µl, Category B CD4 count 200-499 cells/µl, Category C CD4 count <200 cells/µl. According to CDC, AIDS is HIV infection with CD4 count < 200 cells/ml or CD4% < 14%. But WHO staging does not include CD4 count to accommodate poor nations [18].

HIV VIRAL LOAD :

It is a direct measure of in vivo replication of virus and therefore it is a powerful prognostic tool [22]. Measurement of viral load is based on getting the target RNA, Reverse Transcription of RNA into cDNA, PCR amplification of target DNA and detection of dual fluorescent labelled oligonucleotide probes which quantifies HIV-1 target RNA. The test can detect and quantitate HIV-1

RNA as few as 40-50 copies/ml of plasma. Therapy is considered in patients with more than 1,00,000 copies/ml. During therapy, viral load is determined every 3-4 months. Reduction of viral load less than 50 copies/ml in 6 months indicates effective treatment.

Uses :

1. Guide for initiating ART
2. Optimising the duration of treatment.
3. Switching to second line of treatment

TREATMENT[14]:

The main aim of ART is to reduce viral load to improve the quality of life and increase the life span of the patients. HAART regimens helps in preventing opportunistic infection.

Table 2: Goals of ARV therapy[14]
• Clinical goals : Prolongation of life and improvement in quality of life
• Virological goals : Greatest possible reduction in viral load for as long as possible
• Immunological goals : Immune reconstitution that is both quantitative and qualitative
• Therapeutic goals : Rational sequencing of drugs in a fashion that achieves clinical, virological and immunological goals while maintaining treatment options, limiting drug toxicity and facilitating adherence
• Reduction of HIV transmission in individuals : Reduction of HIV transmission by suppression of viral load

Table 3: Initiation of ART, based on CD4 count and WHO clinical staging

WHO Clinical Stage	Recommendations
HIV infected Adults & Adolescents (Including pregnant women)	
Clinical Stage I and II	Start ART if CD4 < 350 cells/mm^3
Clinical Stage III and IV	Start ART irrespective of CD4 count

Classification of drugs :

1. Drugs inhibiting the viral reverse transcriptase enzymes
    a. Nucleoside Reverse transcriptase inhibitors- Zidovudine, didanosine, Zalcitabine, Stavudine, Lamivudine, abacavir.
    b. Non –Nucleoside Reverse transcriptase inhibitors – Nevirapine, Delaviridine, Efavirenz.
    c. Nucleotide Reverse transcriptase inhibitors – Tenofovir.
2. Drugs inhibiting Protease enzyme – Ritonavir, Indinavir, Saquinavir, Amprenavir, Lopinavir, Nelfinavir,Atazanavir, Darunavir.
3. Drugs inhibiting Integrase enzyme – Raltegravir
4. Drugs inhibiting Viral entry – Maroviroc
5. Drugs inhibiting Fusion of viral envelope with host cell- Enfuvirtide.

Principles for selecting the first-line regimen

1. Choose 3TC (Lamivudine) in all regimens
2. Choose one NRTI to combine with 3TC (AZT **or** TDF)
3. Choose one NNRTI (NVP **or** EFV)

DRUG RESISTANCE[13,23]:

Drug resistance is common with monotherapy in HIV infected patient by mutation and natural selection within weeks or months[6].

Resistance to NRTIs is due to two mechanisms which prevent the incorporation of the triphosphorylated NRTIs. The first mechanism is a mutation that allows reverse transcriptase to recognise NRTIs during DNA synthesis thereby preventing their addition in primer DNA chain. The second mechanism is a mutation in the enzyme that enhance the hydrolytic removal of the chain terminating NRTIs, hence continue the DNA synthesis.

Resistance to NNRTIs is by a single mutation in the hydrophobic pocket close to the active site in reverse transcriptase where the NNRTs binds. This binding displaces the catalytic aspartate residue relative to the polymerase binding site hence inhibit HIV-1 replication.

The HIV exhibit resistance to Protease inhibitor is due to mutation in the cleavage sites, causing structural changes which reduce the binding affinity between the inhibitor and mutant protease molecule.

In order to prevent resistant strains to evolve, combination therapy with two or three drugs with different mode of action is give.

Drug resistance can be identified at three levels. First clinically by unresponsiveness with rapid fall in CD4 count and rise in plasma HIV RNA level

in a patient on regular treatment. Second is phenotypic method by viral isolation followed by drug susceptibility testing in cell culture. But it is cumbersome and expensive. Last is genotypic method by using PCR to amplify and screen for viral geneome with common mutation patterns.

HIV DRUG RESISTANCE TESTING [5,16] :

It measures the sensitivity of individual's HIV to different ARV agents which can be measured by phenotypic and genotypic methods. In genotypic assays, sequence analysis of patient's HIV is compared with sequence of virus with known ARV resistance profiles. In phenotypic methods the in vivo growth of the virus is compared with reference strains in the presence and absence of ARV agents.

Uses :

1. Selecting an initial regimen for treatment of new patients
2. Selecting new drugs in drug failure

OPPORTUNISTIC INFECTIONS[2,6]:

BACTERIAL OPPORTUNISTIC INFECTIONS:

1.Tuberculosis(TB):

Of all the opportunistic infection, *Mycobacterium tuberculosis* is the most common serious opportunistic infection and leading cause of morbidity and mortality in HIV infected patients globally[2]. The incidence of TB doubles in the first year following HIV infection and the risk increases with progressive immunodeficiency but can occur in any stage of HIV infection[16,24].

Clinical presentation: Cough for more than 3 weeks which is not responding to antibiotic treatment. Purulent or blood-stained sputum. Night sweats, Weight loss and evening rise of temperature.

Diagnosis: Chest radiography reveals miliary pattern, hilar adenopathy, pleural effusion, focal infiltrates in upper and hilar regions, multilobar infiltrates, Interstitial infiltrates, cavitation with severe immunosuppression, X-ray might appear normal.

Treatment:

Table 4: Treatment Categories and Regimens for Tuberculosis[5]

Category	Type of patients	Treatment Regimens	
		Intensive phase	Continuation phase
Category I	All new pulmonary(smear positive and negative), and extrapulmonary TB cases	2 HRZE	4 HR
Category II	Previously treated cases; relapses or treatment defaulters	2HRZES+ 1HRZE	5 HRE
H= Isoniazid, R=Rifampicin, Z=Pyrazinamide, E=Ethambutol, S=Streptomycin The number before the letters refers to the number of months of treatment			

2.*Mycobacterium Avium* Complex(MAC)[2]: MAC can cause life-threatening symptoms and disseminated disease in advanced HIV disease. MAC is ubiquitous in nature and is found in water, soil and food. It is not seen very commonly in Indian patients with HIV.

Clinical Presentation: A clinical diagnosis is based on MAC syndrome criteria which consists of ≥1 of the following: persistent fever for more than one week, night sweats, diarrhoea, weight loss or wasting, hepatomegaly, splenomegaly, anaemia and alkaline phosphatase more than twice the upper limit of normal.

Diagnosis: X-ray reveals pulmonary infiltrates. The diagnosis is *confirmed* if MAC is isolated from normally sterile body fluid or tissue and is considered *probable* if MAC is isolated from the skin surface, bronchopulmonary, gastrointestinal, or other non-sterile sites along with histopathological confirmation of AFB/MAC.

Treatment: Combination therapy is generally suggested to prevent resistance. Clarithromycin is highly effective while Azithromycin is an excellent substitute in cases of drug interactions or side-effects. Lifelong maintenance therapy is recommmended to prevent recurrence, but may be discontinued when CD4 count rises to >100 cells/µl.

Prophylaxis: Prophylaxis is usually started when CD4 count is <75 cells/µl. Macrolides are effective drugs with risk reduction rates of 70%. Clarithromycin once a day or azithromycin once a week is given as prophylaxis. However, routine prophylaxis for MAC is currently not recommended in India.

3. Bacterial pneumonias[6]: Community acquired bacterial pneumonia is three to fivefold more common in PLHA than among HIV negative individuals. The risk increases with lower CD4 count, intravenous drug use and cigarette smoking.

Clinical presentation: Presents with typical symptoms of pneumonia like fever, productive cough, dypnea and pleuritic chest pain. Physical examination reveals localized pulmonary findings.

Diagnosis: Chest radiography reveals focal infiltrates. Sputum culture is usually positive with multiple polymorphonuclear cells in direct gram stained smear. Leukocytosis and hypoxemia are the other findings. The most common pathogens associated with pneumonia in HIV-infected patients includes S.pneumoniae, H.influenzae, Klebsiella pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa.

Treatment: Second or third generation cephalosporins can be given empirically which can later be streamlined.

Prophylaxis: Optimizing ART is the most effective strategy to prevent recurrence. When the CD4 counts falls below 200 cells/µl, a single dose of 23-valent polysaccharide pneumococcal vaccine should be administered if they have not received it in the preceding five years. Using antibiotics solely to prevent recurrence is not recommended as it may lead to the development of drug-resistant strains and drug toxicity.

4.Bacteremia: Associated with *Salmonella* spp infections or pneumonia. The most common organism isolated are *Staphylococcus aureus* and coagulase negative *Staphylococci* although few gram negative organisms are also seen. Empiric therapy include Vancomycin with aminoglycosides/ β-lactam antibiotics.

FUNGAL OPPORTUNISTIC INFECTIONS[2,6,25]: Fungi are the most common pathogen seen in PLHA but are not common cause of mortality.

1.Candidiasis:

It causes exclusively mucosal infection with oropharyngeal candidiasis seen in about 3/4th of patients with HIV infection. In one-third of the patients it tends to recur and progress in severity with increasing immunodeficiency. Esophageal candidiasis is seen in 20-40% of AIDS patients predominantly with very low CD4 count. Oral Candidiasis is frequently the first indication of immune impairment in HIV-infected patients. The most common candida species causing infection in AIDS patient is *C.albicans* though *C.glabrata*, *C.dubliniensis*, *C.parapsilosis* tend to cause infection in advanced disease.

Clinical Manifestations: Patients with oral candidiasis complain of oral discomfort and classically present with creamy-white plaques on an erythematous base(pseudomembrane form of oral thrush), other manifestations include erythema without plaque(atrophic form) and angular cheilits. Pateints with esophageal candidiasis complain of odynophagia or dysphagia due to ulcers and erosions in the esophagus.

Diagnosis: Clinical Diagnosis is usually based on the characteristic appearance of the lesions along with the ease with which the whitish plaques can be scraped off. The diagnosis is by microscopic demonstration of the yeast forms from scrapings using a 10% potassium hydroxide (KOH) preparation and culture. The oesophageal candidiasis is diagnosed by endoscopic visualization of the lesions along with histopathological demonstration and culture confirmation.

Treatment: Appearance of oral candidiasis is a sign of progressive immunodeficiency and the patient should have their CD4 count measured. If the patient is not on ART, it should be initiated and if already on ART, it should be reassessed. For oral candidiasis either local or systemic therapy can be given but for esophageal candidiasis systemic therapy is indicated. Clotrimazole troche 10 mg five times/day until the lesions resolve (usually 7–14 days) or Nystatin oral suspension 500000 units which is gargled 4–5 times/day may be used topically. Oral medications include fluconazole 100–200 mg/day (150 mg tab x 1–2 times daily) for 7–14 days[(2)].

Prophylaxis: Recurrence is common in about one-third of the patients and most commonly in patients who are not on ART. But prophylaxis is not indicated.

2.Cryptococcosis:

Occurs in patients with very low CD4 count (<50cells/µl). Virtually all Cryptococcosis in HIV infected patient is caused by *Cryptococcus neoformans var neoformans.*

Clinical manifestations: Most common presentation is subacute meningitis or meningoencephalitis with fever, malaise and headache for a period of 2-4weeks. Only one third of patients present with classical signs and symptoms of meningitis, while some present with symptoms of encephalopathy like lethargy, altered sensorium, personality changes and memory loss.

Diagnosis: CSF analysis reveals mildly elevated protein, normal or slightly low glucose, with few lymphocytes. Elevated CSF opening pressure is seen in about 25% of patients. Organism can be demonstrated in CSF by negative staining methods like India ink staining or Nigrosin staining(Capsulated round budding yeast cells), antigen detection by lateral flow assay or latex agglutination test and isolation in culture on Sabouraud Dextrose agar at 37□C. A high titre of more than 1:8 for serum Cryptococcal antigen is presumptive evidence of Cryptococcal infection.

Treatment: Cryptococcal meningitis if untreated becomes fatal. Treatment includes Amphotericin B(0.7mg/kg) given intravenously for 2 weeks followed by maintenance with fluconazole 400mg orally for 8weeks. ART should be initiated and patient should be monitored for development of IRIS.

Prophylaxis: Require long-term treatment with fluconazole 200mg daily until immune function improves with CD4 count >200cells/µl persistently and undetectable plasma HIV RNA.

3.*Pneumocystis jirovecii* Pneumonia:

Previously known as *Pneumocystis carinii*. It is an opportunistic fungal pathogen causing especially pneumonia(PCP) in patients with impaired cell mediated immunity. The initial clue towards the AIDS epidemic were 5 homosexual men who presented with PCP in the absence of previously known immune deficiency disorder. Though the advent of HAART has reduced its incidence, it still remains as an important pathogen causing OI. The risk of acquiring PCP infection increases when the CD4 count reduces beyond 200cell/µl. The rate of relapse following first episode of PCP is high which is approximately 60% within 1 year if no specific prophylaxis or HAART is given to the patient.

Clinical Features: 95% restricted to lung presenting most commonly with progressive dyspnea with dry cough, mild fever and weight loss for 3-4 weeks. On examination, tachypnea is common though lung auscultation may be normal.

Diagnosis: Chest radiography is an important step to guide towards the diagnosis which reveals fine bilateral interstitial and followed by alveolar-interstitial infiltrate progressing from perihilar to peripheral regions. In advanced cases, progressive consolidation with air bronchograms and complete opacification of the lung may be seen. Arterial blood gases must be measured to assess the severity of the disease. Definitive diagnosis requires morphological demonstration of the organism. Bronchoalveolar lavage(BAL) being the reference diagnostic specimen with sensitivity of 95% followed by induced sputum with sensitivity of 50-60%[11,14]. Under high suspicion yet unable to demonstrate the organism in

BAL, transbronchial lung biopsy may help but it has the risk of bleeding and pneumothorax. The various methods used to demonstrate the organism include Methanamine silver stain(gold standard), mucicarmine staining, Giemsa staining, immunofluorescence technique used with fluroscent tagged antibody against *P.jirovecii* and Polymerase chain reaction.

Treatment: Severity of the disease is measured by arterial blood gas as mild (PaO_2 ≥70mmHg), moderate (PaO_2 50-70mmHg) and severe (PaO_2 <50mmHg). Patients with moderate and severe PCP are hospitalized and treated with Trimethoprim-sulfamethoxazole with 15mg/kg/day trimethoprim with 75mg/kg/day sulfamethoxazole in three divided doses. Corticosteroids are also administered in patients with PaO_2 <65mmHg to improve oxygenation, reduce risk of fibrosis and need for mechanical ventilation. Treatment should be given for a period of 21days.

Prophylaxis: Indicated for PLHA with CD4 count <200cells/µl or with history of oropharyngeal candidiasis or any AIDS-defining illness[(26)]. Trimethoprim-sulfamethoxazole is recommended for prophylaxis per day till the CD4 count increases to >200cells/µl and persists for on two consecutive occasions within a period of 6 months[(2)].

4.Microsporidiosis: It is an intracellular organism which had undergone taxonomic change recently and has been re-classified from Protozoa to the Kingdom Fungi[25]. Microsporidiosis is common in PLHA with CD4 count are <100cells/µl.

Clinical Manifestations: The most common presentation is diarrhea, however encephalitis, ocular infection, sinutsitis, myositis and disseminated infection.

Diagnosis: Chromotrope 2R and calcofluor white are few selective stains useful for identification of microsporidia in stoll and other body fluids. Tissue stains like Giemsa, tissue Gram stains (Brown-Hopps Gram stain) and calcofluor white are useful in identification in biopsy specimens[22].

Treatment: Restoration of immune with ART to achieve CD4 count >100cells/µl, correction of dehydration, malnutrition and wasting with fluid support and nutritional supplements wherever applicable.

Prophylaxis: Initiation and optimization of ART to restore immunity in advanced immunodeficiency would prevent the disease

PARASITIC OPPORTUNISTIC INFECTIONS[2,6,27]

1.Cryptosporidiosis:

Protozoan parasite Cryptosporidium, the causative organism infects the small bowel mucosa and presents with diarrhea if symptomatic. CD4 count of <100cells/µl. Common cause of chronic diarrhea in AIDS patients in developing countries with up to 74% diarrheal stool samples demonstrating the organism[28].

Clinical Manifestations: Most commonly presents with acute or subacute watery diarrhea, accompanied by nausea, vomiting and lower abdominal cramping

Diagnosis: Microscopic identification of oocyst in stool with acid-fast staining or direct immunofluorescence. Immunofluorescence is 10 times more sensitive than acid-fast staining[29]. Concentration methods, sedimentation (formalin ether) or floatation methods(Sheather's) may facilitate the diagnosis. Antigen detection with ELISA or immunochromatography can be done. Molecular methods like Polymerase chain reaction (PCR) can detect as low as 5 oocyst in spiked stool sample. Single sample is sufficient for diagnosis in case of profuse diarrheal illness, whereas repeat stool sample is required for milder disease.

Treatment: Restoration of immunity with CD4 cell count to >100cells/µl with ART is sufficient to achieve resolution from clinical cryptosporidiosis[30]. Along with it rehydration and correction of electrolyte imbalance is sufficient.

Prophylaxis: Restoration of immunity with ART is recommended for its prevention.

2. Isosporiasis:

It is caused by the protozoon *Isospora belli* which infects small intestine causing severe diarrhoea and malabsorption. It spreads by food or water contaminated with animal faeces. It causes prolonged, severe diarrhoea and malabsorption when the CD4 count falls to <150cells/µl.

Clinical features: Presents with watery diarrhoea, abdominal pain, flatulence, weight loss, loss of appetite and dehydration are the presenting features.

Diagnosis: The organism is demonstrated by microscopic examination of the stained (Acid Fast Staining) stool sample.

Treatment: Two double-strength TMP–SMX tablets 160/800mg twice daily, or one double-strength tablet q6h daily for 10 days followed by 3 times/day for 3 weeks is the treatment of choice. In cases of sulfa allergy, pyrimethamine and folinic acid may be given for a month.

3.*Toxoplasma gondii* Encephalitis:

It is caused by the protozoa *Toxoplasma gondii* occurring due to reactivation of latent tissue cyst[20]. Risk of infection is high when the CD4 count is <50cells/µl[21].

Clinical Manifestations: Most common presentation is focal encephalitis presenting with headache, confusion, motor weakness and fever which may progress to seizures, stupor, coma and death. Physical examination reveals focal neurological abnormalities.

Diagnosis: Computed tomography (CT) or magnetic resonance imaging(MRI) of brain shows typical multiple contrast-enhancing lesions in grey matter of the cortex or basal ganglia with edema. PLHA patients with Toxoplasmic encephalitis are almost uniformly seropositive for anti-toxoplasma IgG and IgM are usually absent[18].

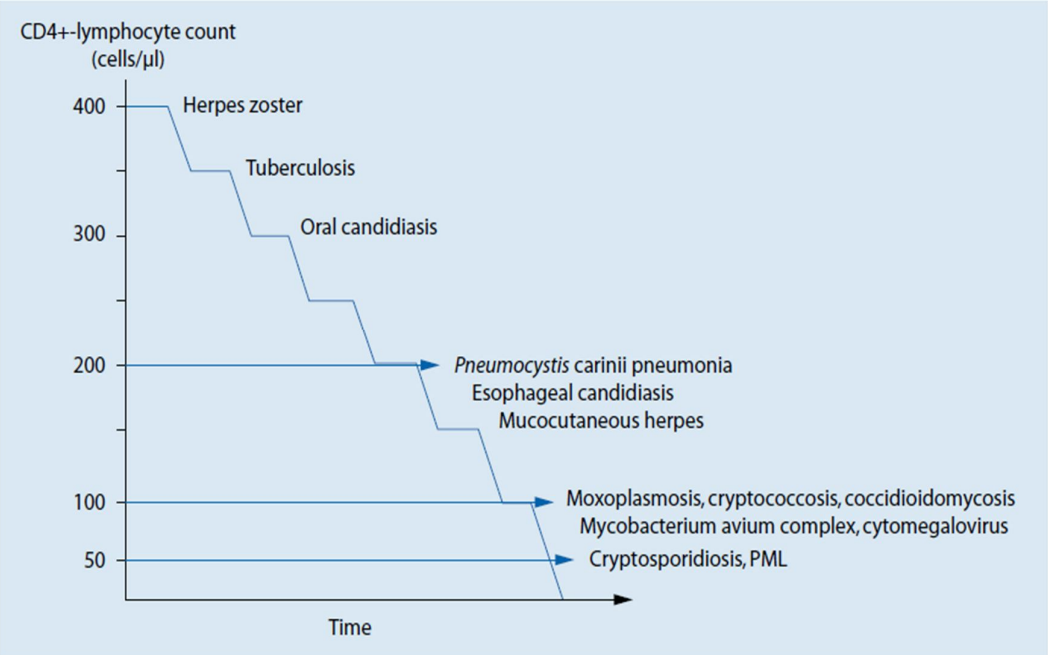
Treatment: Pyrimethamine with sulfadiazine and leucovorin along with PCP prophylaxis[13].

Prophylaxis: Given for patients with CD4 count <100cell/µl. Trimethoprim-sulfamethoxazole(TMP-SMX) double strength 3times weekly until CD4 count increase to >200cells/µl for more than 3months. If the patient is on PCP prophylaxis with TMP-SMX, no additional medication is required for Toxoplasma pophylaxis[13].

CD4 COUNT AND OPPORTUNISTIC INFECTIONS IN HIV INFECTION:

The number of circulating CD4 T-lymphocytes closely correlate with the risk of acquiring several opportunistic infections.

Figure 2: Association between opportunistic infections and CD4 Lymphocyte count[2]



IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME(IRIS)[2]:

IRIS refers to atypical manifestations of OIs with paradoxical clinical deterioration following initiation of HAART. This syndrome is well-described for TB, Cryptococcosis and PCP. It must be distinguished from failure to treatment or prophylaxis, as the management is different.

IRIS is described as an adverse clinical phenomenon following rapid restoration of immune function in a previously severely immunocompromised individual. A temporal relationship with commencement of HAART and exclusion of an alternative explanation must be established.

PLHA recently started on HAART who develop IRD must continue on treatment with both ART and treatment of OI. Steroids are used in life-threatening conditions.

PREVENTION OF OPPORTUNISTIC INFECTIONS[2]:

Primary prophylaxis should be started in all HIV infected patients when they present under WHO Stage III or Stage II with CD4 count <200cells/µl. It may be discontinued when the CD4 cell count is >200cells/µl for 6 months.

Co-trimoxazole (Sulfamethoxazole/trimethoprim) 800mg/160mg PO once daily is the recommended prophylaxis. It is effective in preventing PCP, toxoplasmosis, certain bacterial pneumonias, Nocardiosis and enteric pathogen. In case of allergy to co-trimoxazole, alternative primary prophylaxis against PCP and toxoplasmosis is dapsone 100mg once daily with pyrimethamine 50mg weekly. Desensitization may be suggested for sulfa allergy.

Materials and methods

MATERIALS AND METHODS

STUDY DESIGN : Cross sectional study

STUDY PERIOD : 1 year (July 2016-June 2017)

STUDY SETTING :

The study was conducted in the Institute of Microbiology, in association with Integrated Counselling and Testing Centre, Institute of General Surgery and Institute of Internal Medicine, Madras Medical College & RGGGH.

ETHICAL CONSIDERATION:

All patients satisfying the following criteria were included in the study after obtaining informed written consent in both regional language and English. This study was reviewed and approved by Institutional Ethical Committee with clearance number 17062016. Confidentiality and anonymity were maintained during the study.

SAMPLE SIZE : 140 People living with HIV/AIDS having clinical symptoms and signs suggestive of OI

INCLUSION CRITERIA:

- HIV positive patients more than 18 years of age
- PLHA with clinical symptoms and signs suggestive of OIs

EXCLUSION CRITERIA:

- Patients who did not give consent

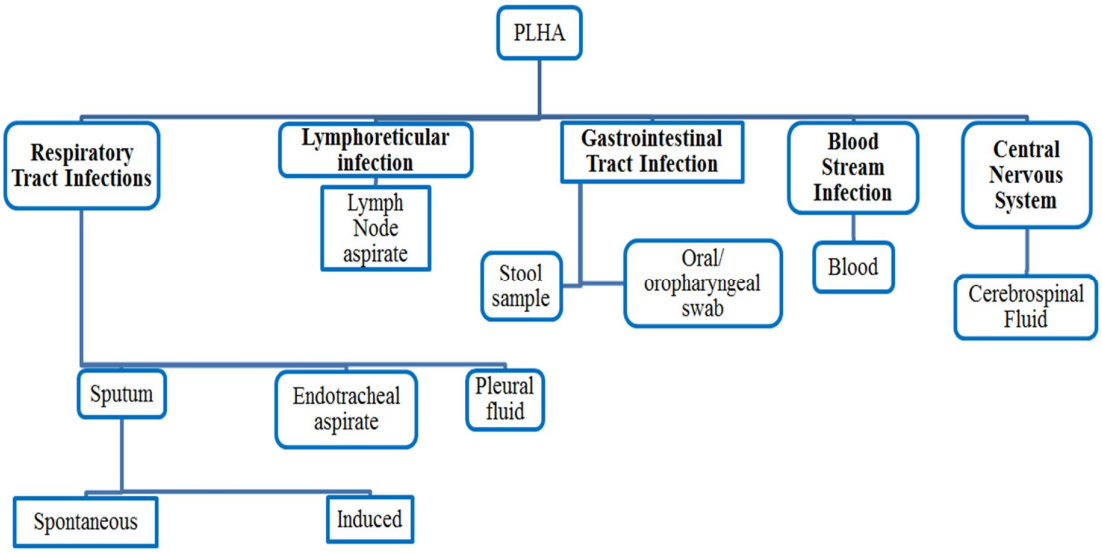
METHODOLOGY:

Confidentiality of HIV status of the patients were maintained at all levels. Personal and demographic data were obtained by structured questionnaire

SAMPLE COLLECTION AND PROCESSING[35,36,37]:

Depending on the patient's clinical features, relevant specimens were aseptically collected and transported to Institute of Microbiology. Observing standard precautions all the specimens were processed for different pathogens as per standard microbiological protocol for identification and/or isolation of pathogens.

Figure 3: Specimens collected



- Whole blood samples were collected from all the patients for CD4 count estimation.

RESPIRATORY SPECIMENS

Samples: Sputum (2 samples-1 spot,1early morning for AFB detection), Pleural fluid

SPUTUM:

1. Early morning sputum was collected after instructing the patients to gargle with water[38].
2. Expectorated sputum from deep cough was collected in a sterile screw capped container and the outside of the container was wiped with phenol containing disinfectant[35].
3. In patients who were unable to produce sputum or with high suspicion of PCP, induced sputum was collected by allowing the patient to inhale aerosolized droplets of 15% sodium chloride solution for 10mins or until a strong cough reflex was initiated[2].
4. Specimen was transported within 2 hours.
5. **Sputum homogenization[38,39]:** The sputum was homogenized with N-acetyl cysteine(NALC) for direct microscopy and culture.

Principle: NALC is a mucolytic agent which has the ability to split the disulfide bonds in the mucoprotein present in the sputum and hence aid in homogenization.

Stock solution prepared:

- 2.94% of Na-citrate in distilled water – autoclaved at 121°C x 15 minutes
- 0.5 g of NALC in 100 ml of Na-citrate (freshly prepared)

- M/15 phosphate buffer (pH 6.8-7.1)

Procedure: -

Specimen was vortexed, equal volume of Sodium citrate and NALC was added to the specimen and vortexed again for 10-30seconds. The mixture was diluted in the phosphate buffer by adding double the volume of mixture and centrifuged at 1000 g for 15 minutes. The sediment was used to prepare smears for direct microscopy and inoculation onto appropriate media for aerobic bacterial and fungal culture.

6. Direct microscopy:

(i) Gram stain[35]: Purulent part of the specimen was used to make a thin smear and Gram staining performed. Quality of the specimen was assessed and rejected if >10squamous epithelial cells/low power field was seen. Smear was examined for polymorphonuclear cells and the predominant bacteria.

(ii) KOH Mount: Sputum was mixed with equal volume of 10% KOH on a clean grease free glass slide, a coverslip was placed over it and observed under low and high power objective of the microscope for the presence of yeast cells or other fungal elements.

(iii) ZEIHL – NEELSEN STAINING :

Purpose: To identify acid fast bacilli in sputum suggesting Mycobacterial infection

- A small strip of blotting paper was placed over the heat fixed smear.

- The filter paper was covered with primary stain, strong carbol fuchsin for 10 minutes with intermittent heating.
- The filter paper was removed and the slide was rinsed with water until the solution runs clear.
- Decolourisation was done with 20% Sulphuric acid for 2 minutes.
- The slide was rinsed with water and the smear covered with counter stain, methylene blue for 3 minutes.
- The slide was rinsed with water and observed under oil immersion objective of the microscope

Table 5: RNTCP Grading of the smears[40]:

No. of bacilli	Result	Grade	No. of fields to be examined
No AFB in 100 oil immersion fields	Negative	-	100
1-9 AFB / 100 oil immersion fields	Scanty	Record exact number	200
10-99 AFB / 100 oil immersion fields	Positive	1+	100
1-10 AFB/ oil immersion field	Positive	2+	50
More than 10 bacilli / oil immersion field	Positive	3+	20

AFB-Acid Fast Bacilli

(iv) Direct immunofluorescent staining for Pneumocystis:

Principle: Works on the principle of direct immunofluorescence with Fluorescent labelled monoclonal antibodies directed against cell wall and matrix antigen of *P.jirovecii* cysts, sporozoites and trophozoites.

Procedure:

i) Purulent part of the induced sputum was taken to make a smear on the slide provided and allowed to air dry

ii) The smear was fixed in acetone for 10 minutes

iii) Using the transfer pipette, 50µl of the detection reagent was placed on each well.

iv) Slide was incubated in humid chamber for 30minutes at $37^{o}C$

v) The slide was washed with water, dried and mounting medium placed over it.

vi) The coverslip was placed and examined using fluorescence microscope.

Results: Atleast two typical cysts with or without honeycomb appearance exhibiting apple green fluorescence was considered positive for the presence of *P.jirovecii.*

7. Culture:

(i) Aerobic Bacterial culture: The homogenised sputum sample was streaked onto MacConkey agar, 5% sheep blood agar and Chocolate agar and incubated at $37^{o}C$ for 18-24hrs with chocolate agar incubated at 5-10% CO_2 in

Candle jar . The plates were observed for growth which were further identified by standard microbiological techniques.

(ii) Fungal culture: The homogenised sputum sample was inoculated onto two Sabouraud's dextrose agar, incubated at 25^{o}C and 37^{o}C and the isolates were further identified by standard microbiological protocol.

8.GeneXpert[(41)]:

- Xpert MTB/RIF cartridge was labeled with the corresponding specimen ID.
- 1ml of expectorated sputum was transferred to a conical, screw-capped tube using a sterile transfer pipette.
- 2ml of Xpert MTB/RIF Sample Reagent (2:1; v/v) was added to the expectorated sputum using a sterile transfer pipette, the lid was replaced and the tube was shaken vigorously 10-20 times.
- The tube was allowed to stand upright for 5 min at room temperature and then the tube was shaken again vigorously 10-20 times
- The tube was allowed to stand upright for another 10 min at room temperature.
- The liquefied sputum sample was then transferred into the open port of the Xpert MTB/RIF cartridge.
- The instrument module door with the blinking green light was opened and the cartridge loaded
- The instrument was set to run and the report was generated.

PLEURAL FLUID:

1. 1.Under aseptic precautions, 3-5ml of pleural fluid was collected by pleural tap. 2% Lignocaine was infiltrated as local anaesthesia, chest wall disinfected with povidone iodine, catheter inserted through the lower border of ribs(6-9th ribs) in the lateral side of chest and fluid collected.
2. Macroscopic appearance of the pleural fluid was observed and recorded.
3. The pleural fluid was centrifuged at 3000rpm per minute, supernatant discarded [37].
4. Direct Microscopy: The smear made with sediment was subjected to Gram staining and AFB staining and interpreted.
5. The sediment was inoculated on Mac Conkey agar, Blood agar and chocolate agar, incubated at 37^{o}C for 18-24hrs, with chocolate agar incubated in candle jar with 5%CO_2.
6. The culture plates were examined for growth which were further identified as per standard microbiological protocol.

STOOL SAMPLES[27]:

1. 2ml of stool sample was collected in a 25ml wide mouthed screw capped container with spoon.
2. Macroscopic examination of the stool was done to look for consistency, presence of mucus, pus, blood and parasite.

3. FORMOL-ETHER SEDIMENTATION TECHNIQUE[27]:

Principle: The faecal material was dissolved in water or solutions of a density below that of the eggs, hence the eggs are concentrated at the bottom.

Procedure(Modified Ritchief's method):

- 1gram of faeces was emulsified in 7ml of 10%formol-saline and kept for 10mins for fixation.
- The mixture was then strained through a wire gauge and filtrate was collected in a centrifuge tube.
- To the filtrate, 3ml of ether was added and mixed vigorously by shaking for 1minute, then centrifuged at 2,000rpm for 2 minutes.
- Four layers were seen in the tube including ether, debris, formalin and sediment from top
- The debris were loosened with a stick, supernatant discarded leaving 1 or 2 drops.
- The deposit was mixed, a drop placed on a glass slide, coverslip placed over it and examined under microscope for presence of parasite.

4. Direct microscopy:

(i) Saline Mount:

A small quantity of faeces was mixed in a drop of saline on a clean glass slide, covered with coverslip and examined under low and high power objective of the microscope for presence of any trophozoites, protozoan cyst, eggs and larvae of helminths.

(ii)Iodine Mount:

Stool was emulsified in a drop of Lugol's iodine(1:5 dilution in distilled water) on a clean glass slide, covered with a clean coverslip and examined under low and high power objective of the microscope. Parasitic forms namely trophozoites, protozoan cyst, eggs and larvae of helminths, when present were identified.

(iii) Modified Acid-Fast staining for Coccidian parasite:

Thin smear from the sediment was made on a clean glass slide, heat fixed it and flooded with carbol fuchsin(4g basic fuchsin+20ml 95% ethanol+8ml phenol+100ml distilled water).

After 9minutes, the slide was washed with water and decolorized with 5% aqueous sulphuric acid for 30seconds.

Washed in tap water and counterstained with 0.3%methylene blue for 1minute. Dried and observed under oil immersion objective of microscope for the presence of acid fast coccidian parasites.

ORAL SWAB[35]:

1. The oral cavity was examined with the use of tongue depressor and the lesion was identified.
2. The lesion was swabbed with two sterile cotton swabs, taking care not to contaminate the swab with saliva.

3. One swab was used for Gram staining and observed for gram positive budding yeast cells with/without pseudohyphae.
4. The other swab was inoculated onto Sabouraud's Dextrose Agar, incubated at 37°C for 24-48 hours and observed for Cream coloured, smooth, pasty colonies.
5. The Candida isolates were identified by Gram staining and other standard techniques like germ tube test, growth in CHROM agar Candida, chlamydospore formation and sugar fermentation test.

BLOOD SAMPLES[36,37]:

1. 10ml of Blood was collected using aseptic technique from ante-cubital vein, inoculated into 50ml Brain Heart Infusion(BHI) broth and incubated at 37^{O}C for 7days.
2. Subcultures were done at 24hrs if there was appearance of turbidity, gas production or presence of microcolonies. Blind subcultures were done at 48hrs and on 7^{th} day of incubation irrespective of appearance of the broth.
3. Subcultures were done on MacConkey agar and 5% sheep blood agar and observed for growth and isolates were identified by standard microbiological techniques.

CSF SAMPLE[35,37]:

1. Under aseptic precaution, lumbar puncture was performed by introducing the spinal needle at the level of L4-L5 vertebra and 1-3ml of CSF was collected in a sterile screw capped tube.

2. Macroscopic appearance including turbidity, blood stained or presence of clot was noted.
3. Sample was centrifuged at 1500rpm for 15 minutes. Supernatant was used for antigen detection and the sediment used for direct microscopy and culture.

4. Direct microscopy:

(i) Gram stain: Heaped up smear was made with the sediment, gram staining performed and observed for presence of pus cells and microorganisms.

(ii) KOH Mount: CSF sediment was mixed with equal volume of 10% KOH on a clean grease free glass slide, a coverslip was placed over it and observed under low and high power objective of the microscope for the presence of yeast cells or other fungal elements.

(iii) India Ink Preparation: One drop of CSF sediment was mixed with equal volume of India ink and coverslip was placed over it to make a thin uniform film. The preparation was then observed under low and high power objective of the microscope for the presence of encapsulated yeast cells.

(iv) Ziehl-Neelsen staining: The sediment was used to make a smear, Ziehl-Neelsen staining was performed and observed for presence of Acid Fast Bacilli.

5. LATERAL FLOW ASSAY FOR CRYPTOCOCCAL ANTIGEN DETECTION:

(i) Principle[18]: It is a dipstick sandwich immunochromatographic assay with gold conjugated monoclonal antibodies against Cryptococcal polysaccharide coated in the form of a line on the strip, which on combining with the cryptococcal antigen present in the sample forms the test band.

(ii) Procedure:

- Two tubes each labelled as positive control and test were placed in a rack.
- One drop of Lateral Flow (LF) specimen diluent was added to each tube.
- To this one drop of CrAg positive control and 40microlitres of supernatant of the test CSF sample were added respectively.
- The white end of the Cryptococcal Antigen Lateral Flow test strip was submerged into the test and control tube each and the strip examined after 10minutes for the presence or absence of test and control band.

(iii) Results:

The presence of two bands(control and test band) regardless of the intensity of the band is considered positive. The test was considered invalid if control band failed to develop.

6.Culture:

(i) Aerobic Bacterial culture: The sediment was inoculated onto MacConkey agar, 5% sheep blood agar and Chocolate agar and incubated at 37^{o}C for 18-24hrs with chocolate agar incubated in candle jar with 5-10% CO_2. The

plates were observed for growth which were further identified by standard microbiological techniques.

(ii) Fungal culture: The sediment was inoculated onto Sabouraud's dextrose agar, incubated at 25^{o}C and 37^{o}C upto 4weeks. The isolates were identified by standard microbiological techniques.

IDENTIFICATION OF MICROORGANISMS:

IDENTIFICATION OF THE BACTERIAL ISOLATES:

The colonies grown on the culture media were further identified by Gram Staining and Biochemical reactions.

Gram stain:-

Using appropriate control strains microscopic examination of Gram stained smears prepared from the isolates was done. Based on Gram reaction, the organism was subjected to a set of biochemical reactions with appropriate quality controls.

Biochemical reactions[35,36]:

For identification of Gram Positive cocci:

1. Catalase
2. Modified Oxidase test
3. Coagulase test
4. Urease test
5. Aminoacid decarboxylation test (Lysine and Ornithine)
6. Arginine dihydrolation test

7. Sugar fermentation test with glucose, lactose, sucrose, maltose, mannose, mannitol, xylose
8. Bile esculin test
9. Differential discs-Novobiocin (5µg), Furazolidone (100µg), Bacitracin (0.04units/disk)

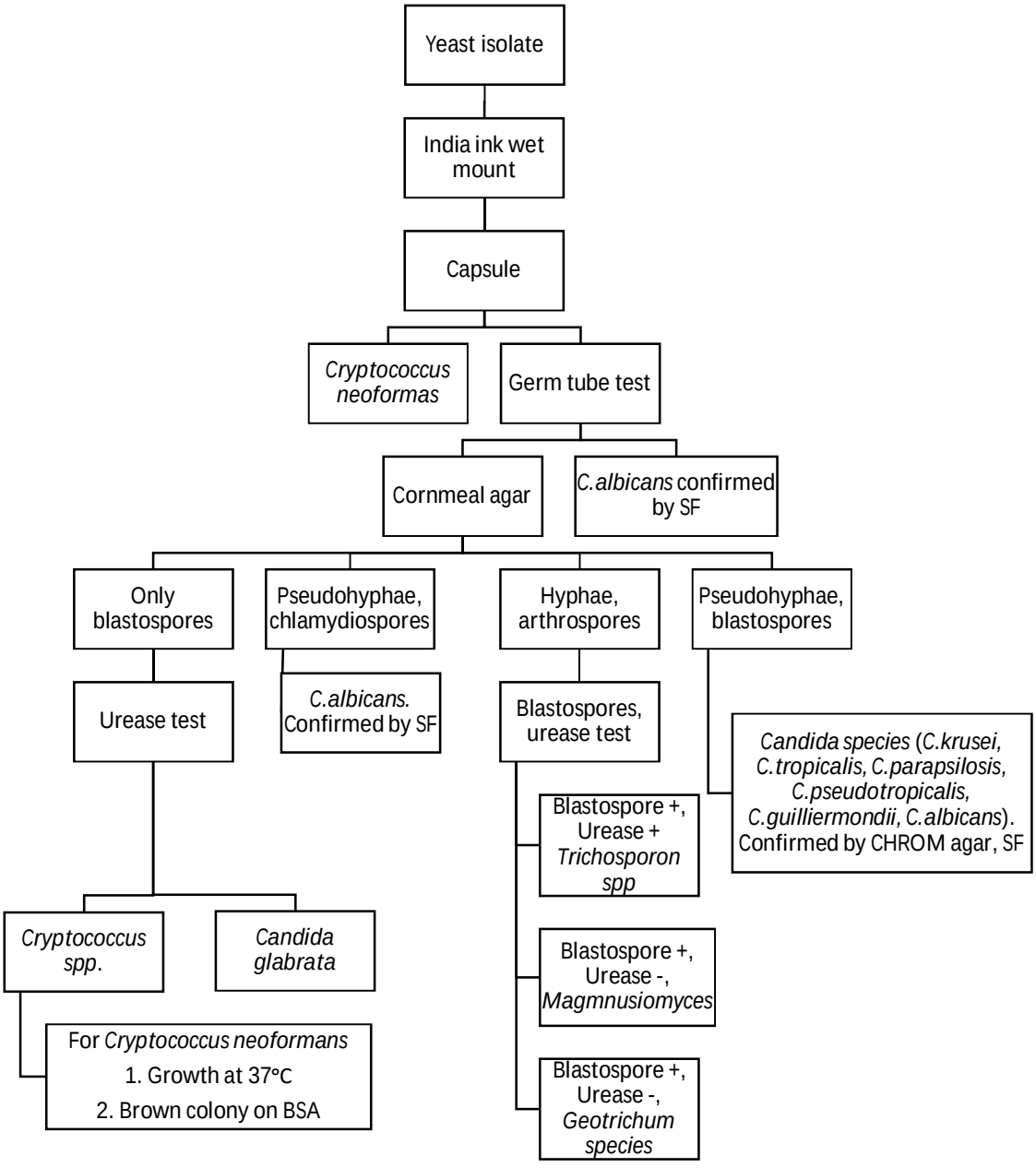
For identification of Gram negative bacilli

1. Hanging drop-to check for motility
2. Catalse test
3. Oxidase test
4. Nitrate reduction test
5. Indole production test
6. Methyl red test
7. Voges Proskaeur test
8. Citrate utilization test
9. Urease test
10. Triple sugar iron agar test
11. Sugar fermentation test Glucose, Lactose, Sucrose, maltose and mannitol
12. Amino acid decarboxylation tests
13. Hugh-Leifson's Oxidation Fermentation test

IDENTIFICATION OF THE FUNGAL ISOLATES[25]:

Gram Stain: Creamy pasty colonies were subjected to Gram staining and morphology of yeast was confirmed. The yeast was further identified by the following algorithm.

Figure 4: Identification of yeast isolates[25]



SF-Sugar fermentation; BSA-Bird Seed Agar

Speciation of Candida isolates:

1. Germ tube(Reynolds-Braude Phenomenon): The test organism was suspended in 0.5ml of sterile serum, incubated at 37^{o}C for 2 hours and a drop from it was transferred on a glass slide. With coverslip placed over the drop, the slide was examined under microscope for presence of germ tube. Germ tubes were seen as long tube like projections extending from yeast cells without constriction at the point of attachment to the cell

2. Cornmeal agar: Cornmeal agar with tween 80 was used for the demonstration of blastospores, chlamydospores and pseudohyphae. A single streak cutting the agar was made with a inoculating straight wire after touching the colony. Three to four streaks were made across the first, a coverslip was placed over it and incubated at 25^{o}C for 3days. The plates were examined without lid under a microscope for chlamydospores, blastospores and pseudohyphae.

3. Fermentation of 2% carbohydrates: For each isolate, six carbohydrate broth including Glucose, sucrose, lactose, maltose, galactose and trehalose were used with 1%peptone, 0.5% sodium chloride and Andrade's indicator(0.005%). Durham's tube was immersed in each tube for detection of gas production. Yeast colonies were inoculated into each broth, incubated at 25^{o}C and examined at 48-72 hours for acid and gas (in Durham's tube) production.

Table 6: Fermentation reactions of Candida species

Sugars	*C.albicans*/ *C.dubliniensis*	*C.tropicalis*	*C.krusei*	*C.parapsilosis*	*C.glabrata*
Glucose	AG	AG	AG	AG	AG
Lactose	-	-	-	-	-
Sucrose	-	AG	-	-	-
Maltose	AG	AG	-	-	-
Galactose	V	AG	-	V	-
Trehalose	V	AG	-	-	AG

A-Acid G-Gas V-variable

4. CHROMagar Candida: The yeast growth from SDA agar was subcultured onto chromogenic medium, incubated at 35^{o}C for 24-48hours and observed for coloured colonies. Different coloured colonies were seen due to reaction between specific enzymes produced by different species and chromogenic substrate in the system.

Table 7: Identification of *Candida* species on CHROMagar

***Candida* species**	**Colour**
C.albicans	Light green
C.dubliniensis	Dark green
C.glabrata	Pink to purple
C.krusei	Pink
C.parapsilosis	Cream to pale pink
C.tropicalis	Blue with pink halo

ANTIMICROBIAL SUSCEPTIBILITY TESTING:

The antimicrobial susceptibility testing of aerobic bacterial isolates were performed as per CLSI guidelines (Clinical Laboratory Standards Institute) M100-S26(2016) and of fungal isolates were performed as per CLSI guidelines M44-A

ANTIBIOTIC SUSCEPTIBILITY TESTING[(42)]:

Three to five identical colonies were picked from an overnight culture with a sterile loop and were suspended in 0.5ml of sterile saline. After matching the suspension with 0.5McFarland turbidity standard, a sterile cotton tipped swab was dipped in the suspension and excess inoculum was drained by pressing it against the sides of the test tube. Lawn culture was made on Muller Hinton agar in three directions by rotating the plate at 60^{o} each time and allowed to dry for 15minutes. The antibiotic discs were placed at equal distance and incubated aerobically at 37^{o}C for 18-24 hours after which the zone of inhibition was measured under transmitted light. The results were interpreted as per the CLSI guidelines.

Table 8: Antibiotic Panel for isolates of the *Enterobactericeae* family and their interpretative criteria.

ANTIBIOTICS	ZONE OF INHIBITION (mm)		
	Sensitive	Intermediate	Resistant
Ofloxacin (5 µg)	≥16	13-15	≤12
Ciprofloxacin (5 µg)	≥21	16-20	≤15
Amikacin (30 µg)	≥17	15-16	≤14
Trimethoprim-Sulphamethoxazole (1.25/23.75 µg)	≥16	11-15	≤10
Cefotaxime (30 µg)	≥26	23-25	≤22
Tetracycline (30 µg)	≥15	12-14	≤11
Imipenem (10 µg)	≥23	20-22	≤19

Table 9: Antibiotic Panel for *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates and their interpretative criteria.

ANTIBIOTICS	ZONE OF INHIBITION (mm)		
	Sensitive	Intermediate	Resistant
Ciprofloxacin (5 µg)	≥21	16-20	≤15
Ofloxacin (5 µg)	≥16	13-15	≤12
Amikacin (30 µg)	≥17	15-16	≤14
Trimethoprim/Sulfamethoxazole (1.25/23.75 µg)	≥16	11-15	≤10
Ceftazidime (30µg)	≥18	15-17	≤14
Tetracycline (30 µg)	≥15	12-14	≤11
Piperacillin-Tazobactam (100/10 µg) for Acinetobacter species	≥21	18-20	≤17
Piperacillin-Tazobactam (100/10 µg) for Pseudomonas aeruginosa	≥21	15-20	≤14
Imipenem(10µg) for Acinetobacter species	≥22	19-21	≤18
Imipenem(10µg) for Pseudomonas aeruginosa	≥19	16-18	≤15

ANTIFUNGAL SUSCEPTIBILITY TESTING[43,44,45]:

Antifungal susceptibility testing for yeast was done by disc diffusion method as per CLSI guidelines. Three to five colonies on SDA were emulsified in 2ml of sterile saline and matched to 0.5McFarland turbidity standards. Lawn culture was made using a sterile cotton swab in three directions on Muller Hinton Agar plate supplemented with 2%glucose and 0.5µg/ml methylene blue, discs were placed on the surface of agar and incubated at 37^{o}C for 24hours. The diameter of zone of inhibition was measured and compared with standard zones interpretive breakpoints published by CLSI M44-A2 guideline.

Table 10: Antifungal panel for *Candida* species and their interpretive criteria

Antifungal drugs	Disc content	Diameter of Zone of inhibition in mm		
		Sensitive	Intermediate	Resistant
Amphotericin B	100IU	≥15	10-14	<10
Nystatin	50µg	≥15	10-14	≤10
Fluconazole	25µg	≥19	15-18(DD)	≤14
Ketoconazole	15 µg	≥28	21-27	≤20
Clotrimazole	10µg	≥20	12-19	≤11
Itraconazole	10µg	≥23	14-22(DD)	<13

DD-Dose Dependent

CD4 DETERMINATION BY PARTEC CYFLOW COUNTER:

The PARTEC CYFLOW Count System is an automated instrument designed for enumerating the absolute cell counts of CD4 in unlysed whole blood.

Principle[18] : Works with the principle of Flow Cytometry. It simultaneously measures and analyses multiple physical and chemical characteristics of single cells as they flow in a fluid stream past optical and/or electronic sensors. It provides information about their relative size, granularity or internal structure and fluoresce in several spectral regions emitted by fluorochrome labeled probes which bind specifically to cellular constituents.

Specimen: Under aspetic precaution,2ml of blood collected in a EDTA vacutainer tube

Procedure: All the procedures were done observing standard precautions

1. The tube containing the sample was mixed by inverting it 10times
2. 20µl of whole blood was transferred to another labeled tube
3. To it 20µl CD4 mAb PE reagent was added and incubated at room temperature in dark for 15minutes.
4. Then 800µl of “no lyse buffer” was added and the tube was mixed.
5. The sample tube was then plugged in the sample port and the start button was pressed to initiate the counting process.
6. After the counting was done, the histogram generated was visualized, CD4 region checked(Gating) and adjusted if necessary.
7. The report of CD4 count was analysed.

Statistical analysis:

The statistical analysis was done using SPSS software. Age was presented as mean ± SD. The categorical variables such as gender and etiological agents were represented as percentages. Comparison of the mean age of the patients with and without opportunistic infections was done using unpaired t test. Comparison of the gender in patients with and without opportunistic infections was done using Chi square test. Comparison of the positivity of GeneXpert and Ziehl Neelsen staining was done using Mc Nemar's Chi square test. Comparison of the opportunistic infections among the different CD4 categories and the WHO staging were done using Chi square test for trends. All P values < 0.05 were considered as statistically significant.

Results

RESULTS

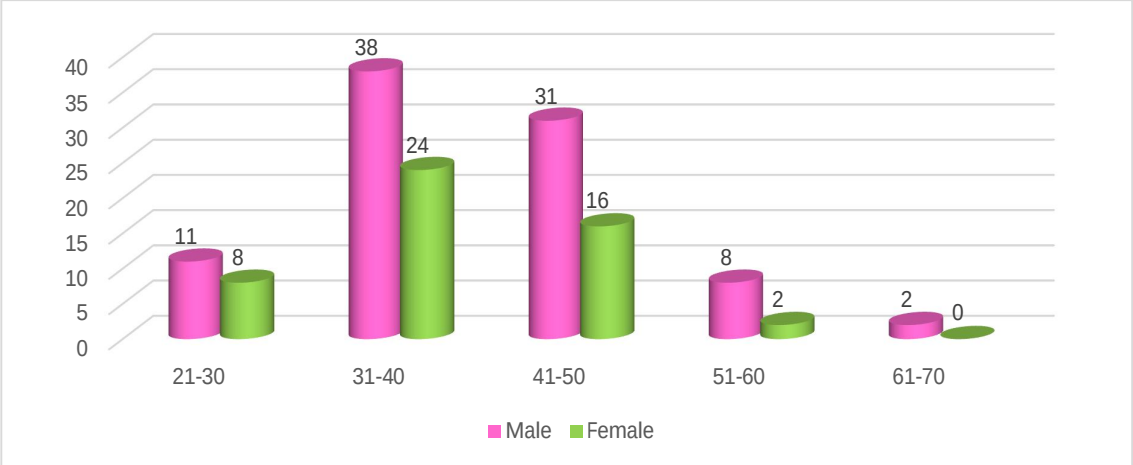
A total of 140 patients were included in this study. Of these 140 patients, 50 (35.7%) patients had one or more than one of the laboratory proven opportunistic infections, while in the remaining 90 (64.3%) the etiological agents were not identified.

Demographic details

Table 11. Frequency distribution of age and gender: n=140

Age range(in ages)	Number of Males	Number of Females	Total
18-20	Nil	Nil	Nil
21-30	11	8	19
31-40	38	24	62
41-50	31	16	47
51-60	8	2	10
61-70	2	Nil	2
Total	90	50	140

Figure 5. Frequency distribution of age and gender:



Age distribution

The mean age of the patients included in this study was 39.76 ± 8.45. The minimum and the maximum age of the patients were 22 and 70, respectively. The mean ages of the patients with Opportunistic infections, with and without laboratory confirmation were 38.60 ± 8.10 and 40.40 ± 8.62, respectively. There was no statistically significant difference in the mean age of the patients with and without laboratory proven opportunistic infections (P value 0.228)

Table 12. Comparison of gender in patients with and without laboratory proven opportunistic Infections (n=140)

		Laboratory proven Opportunistic Infections		Total
		Yes	No	
Sex	F	19 (38.0%)	31 (62.0%)	50 (35.7%)
	M	31 (34.4%)	59 (65.6%)	90 (64.3%)
Total		50	90	140

Gender distribution

Of the 140 patients, 50 (35.7%) were females and 90 (64.3%) were males (Table12). Among the 50 female patients with HIV, 19 (38.0%) had laboratory proven opportunistic infections. Similarly among the 90 male patients with HIV, 31 (34.4%) had laboratory proven opportunistic infections. There was no statistically significant difference in the prevalence of opportunistic infections between female and male patients (P value 0.813)

Specimens

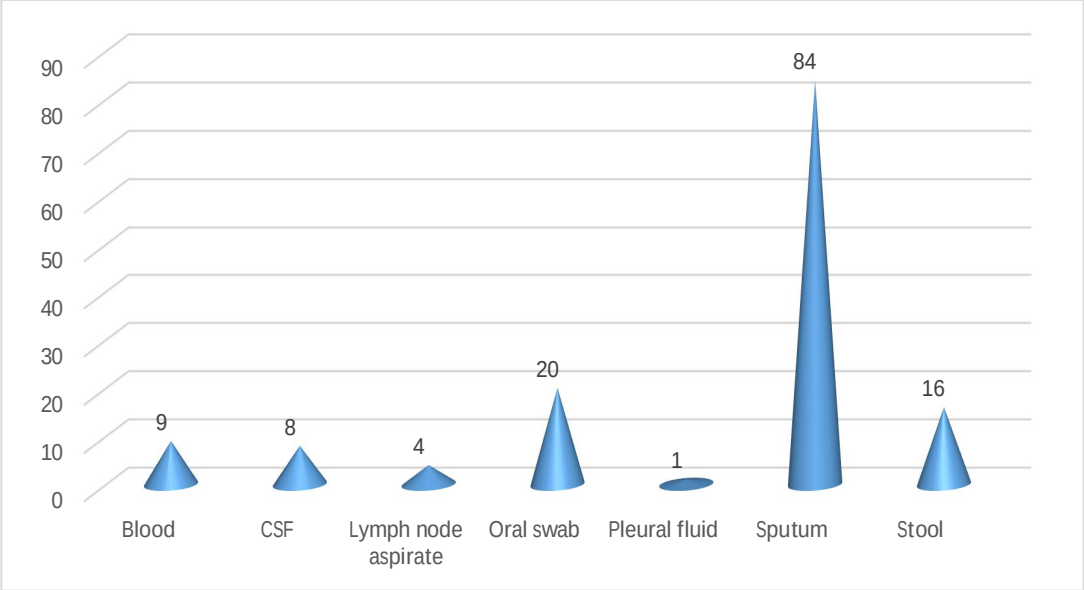
A total of 142 specimens were collected from the study patients based on their clinical symptoms (Table 13).

Table 13. Frequency distribution of the specimens (n=142)

Type of specimen	No. of samples
Blood	9
CSF	8
Lymph node aspirate	4
Oral swab	20
Pleural Fluid	1
Sputum	84
Stool	16
Total	**142***

* - In one patient both sputum and CSF were collected and in another patient both sputum and oral swab were collected.

Figure 6: Frequency distribution of the specimens



Opportunistic infections

Of the 140 patients, 50 (35.7%) patients had laboratory proven opportunistic infections. The etiological agents of the opportunistic infections are summarised in Table 16. 48 patients had one opportunistic infection each. Two patients had dual opportunistic infections. One patient had Pulmonary tuberculosis and Oral Candidiasis while another patient had Tuberculous Meningitis and Bronchopneumonia caused by *Klebsiella pneumoniae*. The distribution of bacterial, fungal and parasitic causes of opportunistic infections is depicted in Figure 7.

Figure 7. Distribution of bacteria, fungi and parasitic causing opportunistic infections (n=52)

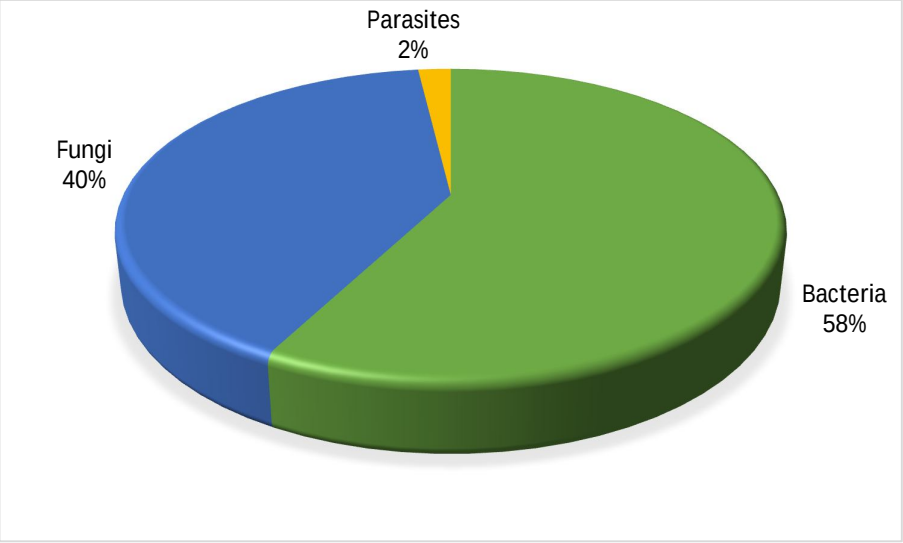


Table 14. Profile of Bacterial pathogens causing OIs: (n=30)

Clinical Diagnosis	Samples	Pathogen	No. of pathogen	Percentage (%)
Pulmonary Tuberculosis	Sputum	*Mycobacteria*	21	70
Bronchopneumonia	Sputum	*K.pneumoniae*	4	13.3
Bronchopneumonia	Sputum	*P.aeruginosa*	1	3.3
Meningitis	CSF	*Mycobacteria*	3	10
Cold abscess	Lymph node aspirate	*Mycobacteria*	1	3.3

Figure 8: Profile of Bacterial pathogens causing OIs

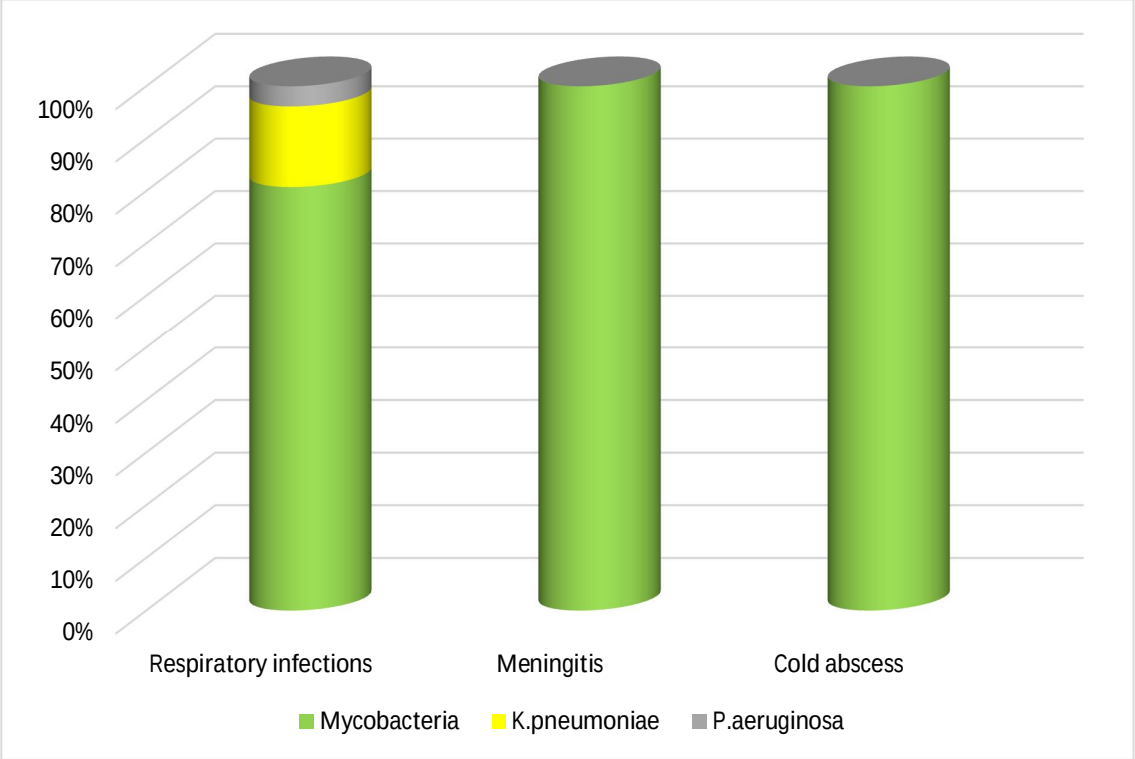
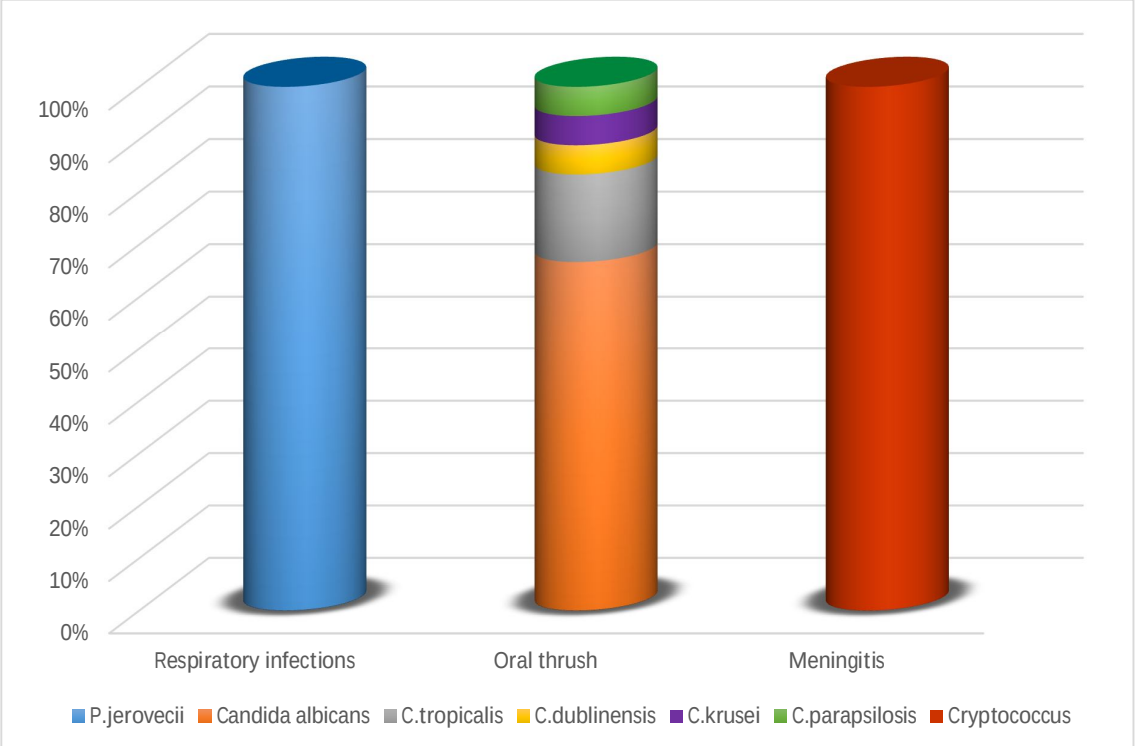


Table 15. Profile of Fungal pathogens causing OIs(n=21)

Clinical Diagnosis	Samples	Pathogen	No. of pathogen(n=21)	Percentage (%)
Bronchopneumonia	Induced sputum	*P.jirovecii*	1	4.7
Oral thrush	Oral swab	*Candida albicans*	12	57.1
		Candida tropicalis	3	13.3
		Candida dubliniensis	1	4.7
		Candida krusei	1	4.7
		Candida parapsilosis	1	4.7
Meningitis	CSF	*Cryptococcus neoformans*	2	9.5

Figure 9:Profile of Fungal pathogen causing OIs (n=21)



Profile of Parasite causing Opportunistic infections:

Among the 52 patients with opportunistic infections, *Strongyloides stercoralis* was demonstrated in stool sample of 1 patient with diarrhea.

Table 16. Cumulative profile of etiological agents causing opportunistic infections (n=52)

	Frequency	**Percentage (%)**
Bacteria		
Mycobacteria	25	48.08
Klebsiella pneumoniae	4	7.69
Pseudomonas aeruginosa	1	1.92
Fungi		
Candida albicans	12	23.08
Candida dubliniensis	1	1.92
Candida krusei	1	1.92
Candida parapsilosis	1	1.92
Candida tropicalis	3	5.77
Cryptococcus neoformans	2	3.85
Pneumocystis jirovecii	1	1.92
Parasite		
Strongyloides stercoralis	1	1.92
Total	**52**	

Bacterial opportunistic infections were the most common OIs seen in PLHA of which *Mycobacterium tuberculosis* was the most predominant pathogen responsible for 48% of opportunistic infections followed by fungal opportunistic infections by *Candida* species accounting for 34%. Among the *Candida* species, *Candida albicans* was the most common species responsible for OI.

Figure 10: Cumulative profile of etiological agents causing opportunisic infections

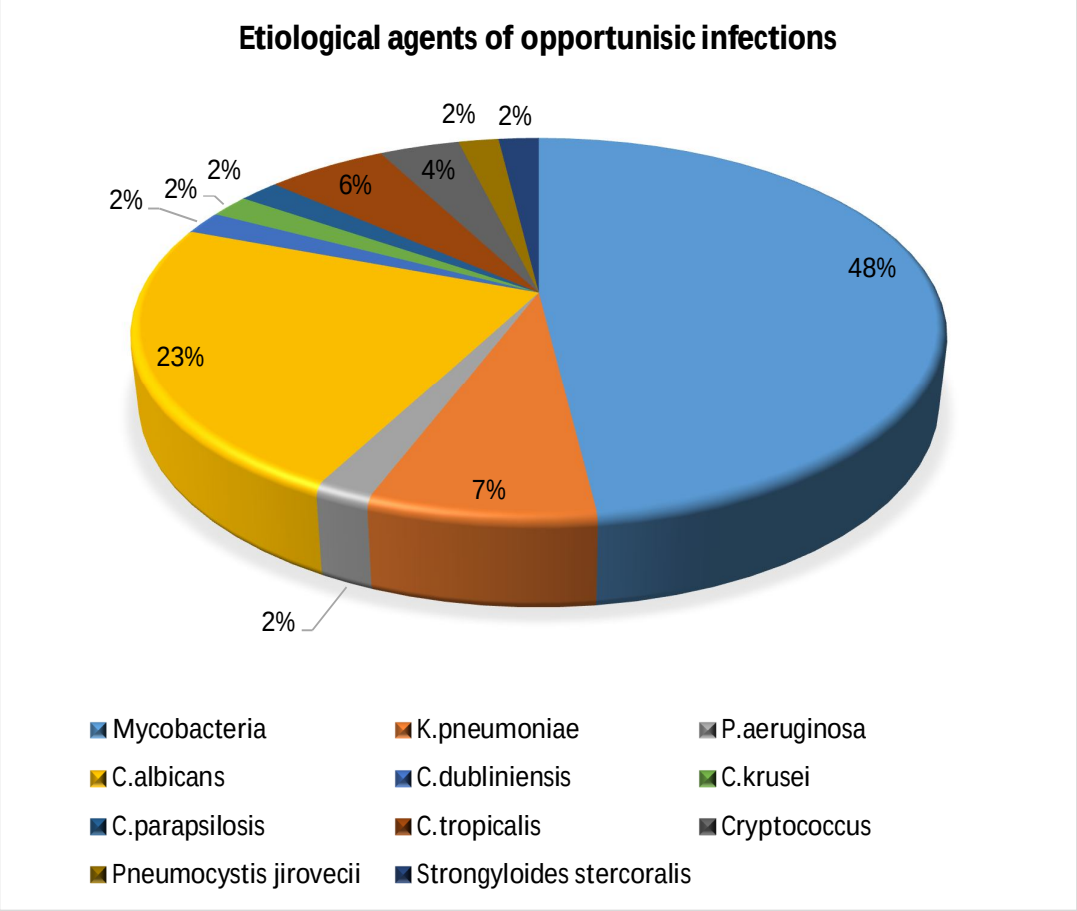


Table 17. Sample wise distribution of *M.tuberculosis*(n=25)

Sample (n=25)	Frequency	Percentage
Sputum	21	84%
Lymph Node	1	4%
CSF	3	12%

Among tuberculosis infection, Pulmonary tuberculosis was more common (84%) compared to extrapulmonary tuberculosis (16%). Among extrapulmonary tuberculosis, tuberculous meningitis was more common

Figure 11:Sample wise distribution of *M.tuberculosis*

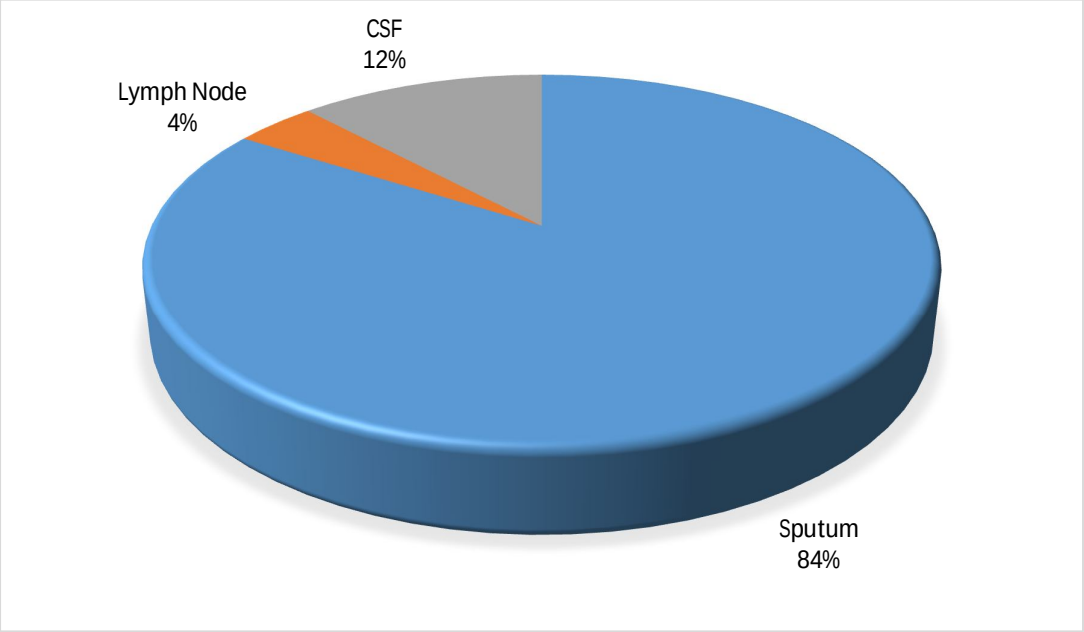


Table 18. Comparison of the positivity of GeneXpert and Ziehl Neelsen staining (n=37)

		GeneXpert		Total
		Positive	Negative	
ZN	Positive	12 (32.4%)	0 (0.0%)	12 (32.4%)
	Negative	5 (13.5%)	20 (54.1%)	25 (67.6%)
Total		17 (45.9%)	20 (54.1%)	37

Out of the 37 samples (Sputum, CSF and Lymph node aspirate) tested, 17 (45.9%) were positive for *M. tuberculosis* by GeneXpert, while only 12 (32.4%) were positive by ZN staining. GeneXpert contributed to detection of additional 5 cases of TB. The difference in the positivity of GeneXpert and ZN staining was only marginally significant (P value 0.063).

Table 19. Antibiotic susceptibility Pattern of Bacterial Pathogens (n=5)

Organism	AK	OF	CIP	COTRI	CTX	CAZ	PT	TET	IMP
K.pneumoniae (n=4)	100%	100%	75%	75%	50%	-	-	100%	100%
P.aeruginosa (n=1)	100%	100%	100%	-	-	100%	100%	-	100%

AK-Amikacin; OF-Ofloxacin; CIP-Ciprofloxacin; CTX-Cefotaxime; CAZ-Ceftazidime; PT- Piperacillin-tazobactam; TET- Tetracycline; IMP-Imipenem

- Of the 4 *Klebsiella pneumoniae* isolates, 2 were Extented Spectrum β-Lactamase (ESBL) producers
- All the *M.tuberculosis* detected by GeneXpert from the 17 samples were sensitive to Rifampicin

Table 20. Antifungal susceptibility Pattern of Fungal Pathogens(n=20)

Fungal species	Ampho tericin B	Nystatin	Fluco nazole	Keoto conazole	Clotri mazole	Itra conazole
Candida albicans (n=12)	100%	100%	91.6%	91.6%	100%	91.6%
Candida tropicalis (n=3)	100%	100%	100%	100%	100%	100%
Candida dubliniensis (n=1)	100%	100%	100%	100%	100%	100%
Candida krusei (n=1)	100%	100%	-*	100%	100%	100%
Candida parapsilosis (n=1)	100%	100%	100%	100%	100%	100%
**C.krusei* is intrinsically resistant to Fluconazole						

Among the 12 *Candida albicans* tested, 10 isolates were susceptible to all the antifungals. Out of the two resistant isolates, one was resistant to ketoconazole and Flucanazole, and the other was resistant to Itraconazole

Table 21. Association of WHO stage and opportunistic infections(n=140)

		Opportunistic Infections		P value
		Yes	No	
WHO Stage	II	2 (12.5%)	14 (87.5%)	0.091
	III	14 (34.1%)	27 (65.9%)	
	IV	34 (41.0%)	49 (59.0%)	
Total		50	90	

The prevalence of opportunistic infections showed an increasing trend with increase in WHO stage.

Table 22. Association of CD4 count and opportunistic infections (n=140)

		Laboratory proven Opportunistic Infections		P value
		Yes	No	
CD4 Cell Count	<200	39* (50.6%)	38 (49.4%)	<0.0001
	200 - 499	10 (23.3%)	33 (76.7%)	
	≥ 500	1 (5.0%)	19 (95.0%)	
Total		50	90	
*Out of 39, 2 patients had 2 laboratory proven opportunistic infections				

The CD4 cell counts were analyzed according to CDC category and correlated with opportunistic infections. The prevalence of opportunistic infections showed an increasing trend with decrease in CD4 count. There was significant association between CD4 count and the prevalence of opportunistic infections (P value <0.0001).

Figure 12:Association of CD4 count and opportunistic infections

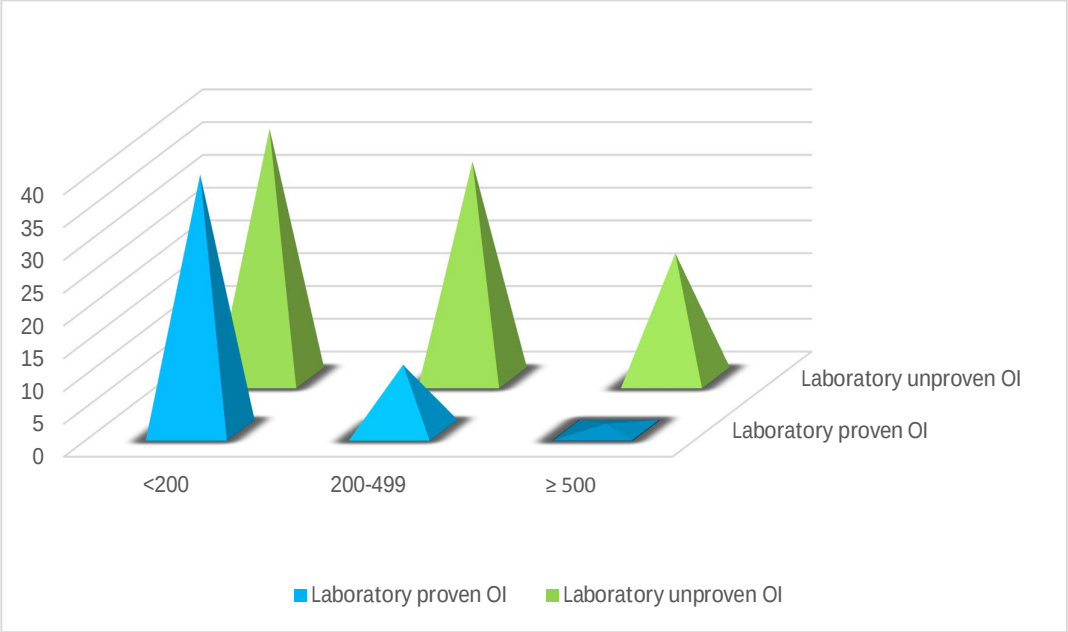


Table 23. Association of CD4 count and candidiasis (n=140)

		Candidiasis		P value
		Yes	No	
CD4 Cell Count	<200	16 (20.8%)	61 (79.2%)	0.007
	200 – 499	2 (4.7%)	41 (95.3%)	
	≥ 500	0 (0%)	20 (100.0%)	
Total		18	122	

There was significant association between CD4 count and the prevalence of candidiasis (P value 0.007). Of the 18 patients with candida infections, 16 patients had CD4 count < 200 cells/ µl, while 2 patients had CD4 count between 200 – 499 cells/ µl. Candida infection was not found in the 20 patients with CD4 count ≥ 500 cells/ µl.

Figure 13: Association of CD4 count and candidiasis (n=140)

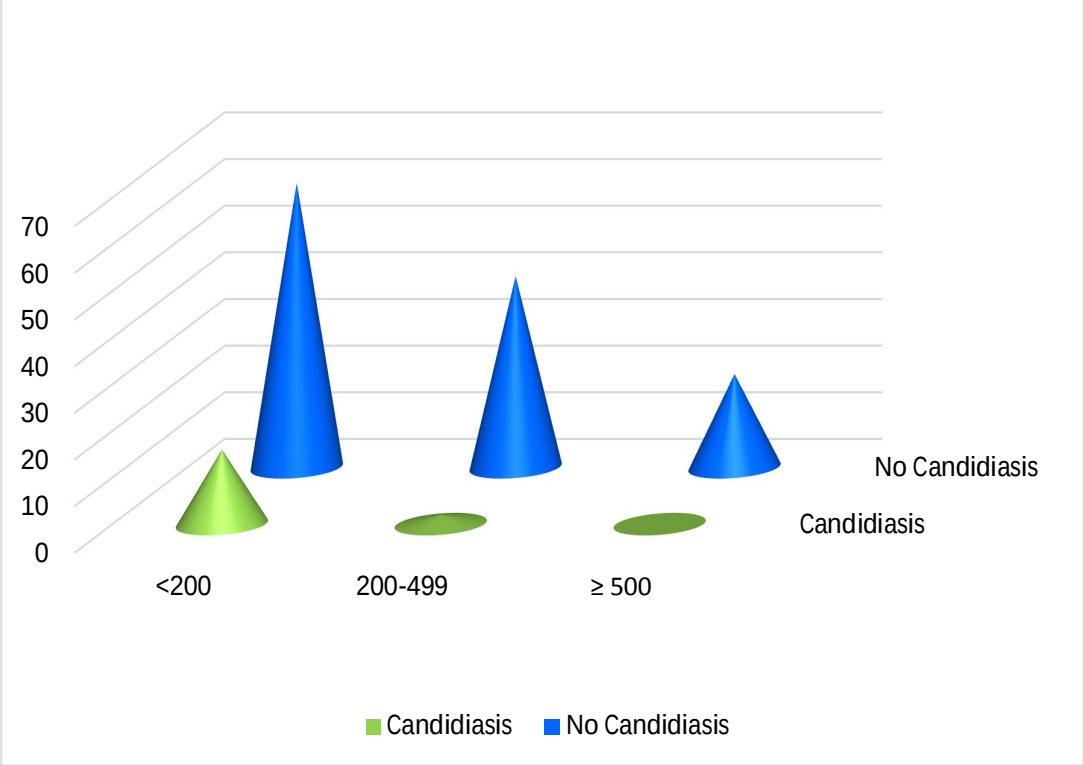
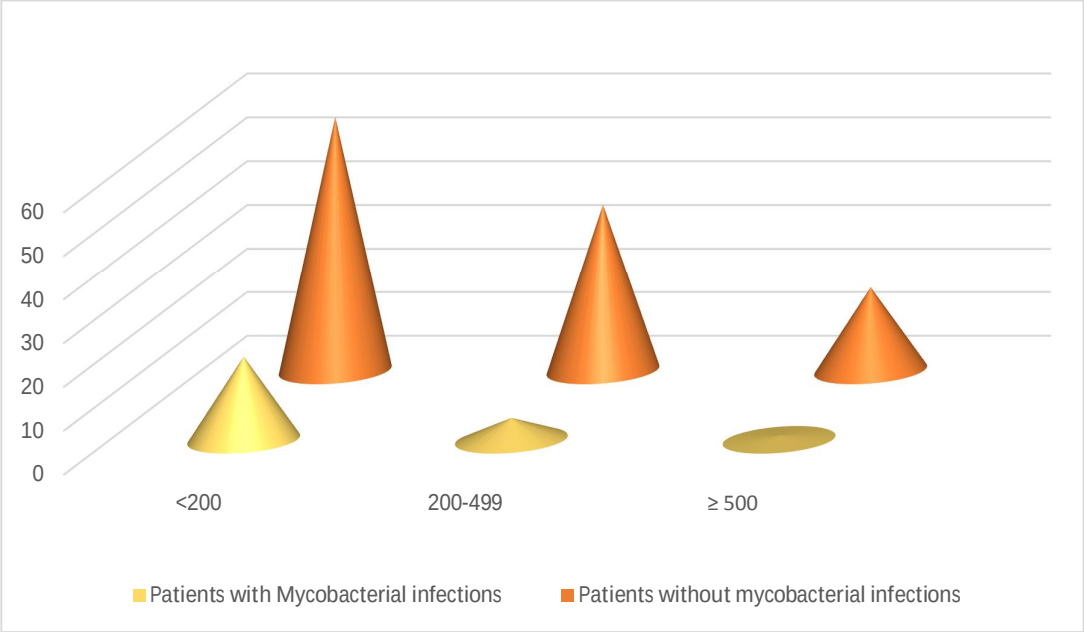


Table 24. Association of CD4 count and Mycobacterial infections (n=140)

		Mycobacterial Infection		P value
		Yes	No	
CD4 Cell Count	<200	19 (24.7%)	58 (75.3%)	0.05
	200 - 499	5 (11.6%)	38 (88.4%)	
	≥ 500	1 (5.0%)	19 (95.0%)	
Total		25	115	

The prevalence of Mycobacterial infections showed an increasing trend with decrease in CD4 count and this association was significant (P value 0.05)

Figure 14: Association of CD4 count and Mycobacterial infections



Association of CD4 count and other opportunistic infections

The CD4 count of the 2 patients with Cryptococcal meningitis was 32 and 210 cells/ µl. The CD4 count of the patient with *Pneumocystis jirovecii* Pneumonia was 34 cells/ µl. The CD4 count of the patient with *Strongyloides stercoralis* infection was 41 cells/ µl. Out of the 4 patients with *Klebsiella pneumonia* causing bronchopneumonia, 3 had CD4 count <200 cells/ µl, while one patient had a count of 258 cells/ µl.

Discussion

DISCUSSION

In People living with HIV/AIDS, the morbidity and mortality is mainly due to opportunistic infections which take advantage of the lowered cellular and humoral defence mechanisms of the patient. The infections encountered in PLHA vary widely including those caused by bacteria, fungi, viruses and parasites. Tuberculosis (TB) is the most common life-threatening opportunistic infection affecting PLHA as per the World Health Organisation(WHO) report 2015 particularly in India[46,2].

AGE DISTRIBUTION:

The mean age of the patients included in the present study was 39.76±8.45 years.

GENDER DISTRIBUTION:

Though males(64.3%) were predominant in the study group, there was no statistically significant gender difference between laboratory proven and unproven cases with opportunistic infection.

OPPORTUNISTIC INFECTIONS:

In the present study, Tuberculosis(48%) was observed to be the most frequent opportunistic infection followed by Candidiasis(34.6%), Bronchopneumonia caused by *Klebsiella pneumoniae* (7.7%), Cryptococcal meningitis(3.85%), Bronchopneumonia caused by *Pseudomonas aeruginosa*

(1.9%), *Pneumocystis jirovecii* Pneumonia (1.9%) and *Strongyloidosis stercoralis* (1.9%).

Despite being preventable and curable, tuberculosis (TB) is the leading cause of death from infectious disease globally, with nearly 10 million people developing TB and 1.5 million people dying from TB in 2014[46]. TB is the leading cause of morbidity and mortality among people living with HIV worldwide, with 1.2 million new HIV-infected persons reported with TB and 3,90,000 deaths in 2014[6]. Tuberculosis was the most prevalent OI reported in this study accounting to 48%, similar to the studies done by Vandana Dabla, et al. at Delhi 2014 and Shahapur, et al. at Karnataka 2009 who reported the prevalence of 28.7% and 43.6% respectively whereas Sangeeta, et al. at Ahmedabad reported Candidiasis as predominant pathogen with the prevalence of 32.67% followed by Tuberculosis(22.71%)[47,48,49)]. Candidiasis being a frequent infection among PLHA, has been documented in upto 70% of the patients infected with HIV[2]. In the current study, the second most common OI among PLHA was Candidiasis with a prevalence of 34.61% similar to the study by Vandana Dabla, et al. who reported Candidiasis as the second most common OI[47].

BACTERIAL OPPPORTUNISTIC INFECTIONS:

Bacterial infection was seen most commonly in patients with respiratory tract infections. Diseases like bacterial pneumonia occur at a higher rate in HIV infected people than in general population[50]. In this study *M.tuberculosis* (83.3%) was the commonest bacterial pathogen causing OI followed by *Klebsiella*

pneumoniae (13.3%) and *Pseudomonas aeruginosa* (3.3). This finding was similar with the observation of Sangeeta, et al. Ahmedabad where *M.tuberculosis* followed by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were the common bacterial opportunistic pathogens in PLHA though there was a slight difference in their distribution namely *M.tuberculosis* (60.5%), *Klebsiella pneumoniae* (15.7%), *Pseudomonas aeruginosa* (10.5%), *S.aureus* (7.8%) and *Escherichia coli* (1.9%)[49].

FUNGAL OPPORTUNISTIC INFECTIONS:

The most common fungal pathogen causing OI in PLHA was *Candida species*(85.7%) followed by *Cryptococcus species* (9.5%) and *Pneumocystis jirovecii* (4.7%).

The Candida species were isolated from Oral thrush in PLHA. Oral Candidiasis is a common oral manifestation in PLHA and is the second most common opportunistic infection in PLHA from India[2] as with the present study, though in study done by Sangeeta, et al., Candidiasis was reported as the most common OI. Out of 18 Candida species isolated from oral lesions, 12 were *Candida albicans*(66.6%) while 6 were *non albicans Candida species*(33.3%) which were consistent with other studies done by Sangeetha, et al., Nadeem, et al., Francis, et al. and Kaur, et al.[49,51,52].

Cryptococcus neoformans was isolated from 2 out of 5 PLHA with meningitis. In the present study Cryptococcus infections is the second most

common fungal infection accounting to 9.5%. There were no Pulmonary Cryptococcosis recorded in this study.

Though PCP is one of the most common opportunistic infection in PLHA living in industrialised nation, its prevalence is rare in tropical and developing countries[53]. In the present study the prevalence of PCP was 1.92%.

PARASITIC OPPORTUNISTIC INFECTIONS:

In the present study, 16 patients presented with mild to moderate diarrhoea. *Strongyloides stercoralis* was demonstrated from one patient whose CD4 count was 41cells/µl.

TUBERCULOSIS

According to the WHO report 2015, TB has become one of the leading cause of death among HIV infected patients killing almost three fourth of affected cases[54]. In the current study, out of 25 cases reported as TB, 84% were Pulmonary infection while 16% were extrapulmonary infection similar to the findings in Ajay kumar, et al., from South India[55] though Kumarasamy, et al. in his study reported higher prevalence of extra pulmonary tuberculosis than pulmonary which is contrary to this study[56].

Comparison of positivity of GeneXpert and Ziehl Neelsen staining: Monika, et al. estimated the sensitivity and specificity of GeneXpert were 86.8% and 93.1% respectively while that of ZN staining were 22.2% and 78.5%[57]. In the present study 37 samples(including Sputum, CSF, Lymph node aspirate) with suspected TB were subjected to both GeneXpert and Ziehl Neelsen(ZN) staining.

Of these samples 45.9% were positive by GeneXpert, while only 32.4% were positive by ZN staining.

All the *M.tuberculosis* detected by GeneXpert (17) were sensitive to Rifampicin

ORAL CANDIDIASIS:

Among the 18 Candida isolated from Oral thrush of PLHA, *Candida albicans* was the most frequently isolated species accounting to 66.6%, followed by *C.tropicalis* (16.6%), *C.dubliniensis* (5.5%), *C.krusei* (5.5%) and *C.parapsilosis* (5.5%).

Isadora, et al. Mexico, demonstrated the same with *C.albicans*(71.8%) being the most common species followed by *C.tropicalis*(12.2%), *C.glabrata*(8.3%), *C.parapsilosis*(2.2%), *C.krusei*(1.7%) and *C.guilliermondii*(1.1%) [(58)].

Faseela, et al., Karnataka[(59)], the study done with 40 Candida isolates from oral thrush 60% were *C.albicans* and was the predominant species, followed by 15% *C.tropicalis*, 10% *C.glabrata*, 5% *C.dubliniensis*, 5% *C.krusei*, 2.5% *C.lusitaniae* and 2.5% *C.parapsilosis* which was similar to the findings in the present study.

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIAL PATHOGEN:

Among the 4 *Klebsiella pneumoniae* isolated from sputum sample of PLHA with bronchopneumonia, 2 were Extended Spectrum Betalactamase producers while the other 2 *Klebsiella pneumoninae* and the *Pseudomonas aeruginosa* were susceptible to all the antibiotic drugs tested as per CLSI guidelines.

ANTIFUNGAL SUSCEPTIBILTY PATTERN OF FUNGAL PATHOGEN

Though *Candida albicans* was the most frequent pathogenic species isolated from oral lesions, other non-*albicans Candida* species have become a significant cause of infection in PLHA in this study. The clinical importance of these non-*albicans Candida* species lies in the fact that they are usually less susceptible to the more commonly used azole antifungal drugs[25].

The Antifungal susceptibility test performed in this study revealed that among 12 *C.albicans*, 10 isolates were sensitive to all the antifungals tested while 2 were resistant of which, 1 isolate was resistant to Fluconazole and Ketaconazole and the other isolate was resistant to itraconazole. All the non-*albicans Candida* isolates were sensitive to all the antifungals tested.

In the study done by Faseela, et al., in Karnataka, she demonstrated that 29% of *C.albicans* were resistant to Fluconazole and 1 *C.tropicalis* showed resistance to Amphotericin B while others were sensitive[59].

WHO STAGING AND OPPORTUNISTIC INFECTION:

The Clinical staging is based on the clinical findings that aid in the diagnosis, evaluation, and management of HIV/AIDS. It does not require CD4 cell count, hence is used in countries to determine eligibility to initiate ART and assess the severity of the disease especially in the settings where CD4 testing facility is not available.

In the present study, the prevalence of opportunistic infections showed a proportional increase with increase in WHO clinical Stage though the association lacked to demonstrate any statistical significance.

ASSOCIATION OF CD4 COUNT AND OPPORTUNISTIC INFECTIONS:

In the current study, the prevalence of opportunistic infections showed an increasing trend with decrease in CD4 cell count which was statistically significant having the mean CD4 count of 243cells/µl in PLHA with OI being. This finding depicts that depletion of CD4 cell results in incidence of more opportunistic infection.

ASSOCIATION OF CD4 COUNT AND CANDIDIASIS:

The mean CD4 cell count in PLHA with Candidiasis was 139cells/µl. There was significant association between decrease in CD4 count and prevalence of Candidiasis in the present study. Most of the studies including Ajay kumar, et al.,[55] reported candidiasis in PLHA at CD4 count <200cells/µl[9] which is same as the findings in the present study except 2 patients in whom Candidiasis was seen at CD4 count 328 and 210 cells/µl.

ASSOCIATION OF CD4 COUNT AND MYCOBACTERIAL INFECTIONS:

TB incidence doubles in the first year following HIV infection and can occur at any CD4 cell count, though the risk increases with progressive immunodeficiency[6]. Pulmonary tuberculosis is more common at CD4 count between 200-500cells/µl while disseminated and extrapulmonary TB at less than <200cells/µl[2].

In the present study also the prevalence of tuberculosis showed an increasing trend with decrease in CD4 cell count and the association was analysed to be significant. The mean CD4 count observed in cases of Pulmonary tuberculosis and extrapulmonary tuberculosis were 146cells/µl and 231cells/µl which was on par with the findings of Ajay kumar, et al., and Shahapur, et al[55,60].

MORTALITY:

Mortality was observed in 3 patients included in the study where 2 patients had Tuberculous Meningitis and the other one diagnosed with Cryptococcal meningitis.

Summary

SUMMARY

- 140 PLHA with symptoms of OIs were included in the study after informed written consent.
- Of the 140 PLHA recruited, etiological agents causing opportunistic infection was identified in 50 PLHA with two patients showing dual infection.
- Among the opportunistic infections identified, Tuberculosis was the predominant OI in PLHA with the prevalence of 48% followed by Candidiasis(34.6%), Bacterial bronchopneumonia (9.6%), Cryptococcal meningitis(3.85%), *Pneumocystis jirovecii* pneumonia (1.9%) and Strongyloidosis(1.9%).
- In the present study, Pulmonary tuberculosis was more common than extrapulmonary tuberculosis.
- GeneXpert was more sensitive in detecting tuberculosis infection compared to Ziehl Neelsen staining.
- The *Mycobacterium tuberculosis* identified by GeneXpert were all sensitive to Rifampicin.

- Among the 4 *Klebsiella pneumoniae* isolated from sputum sample of patients with bronchopneumonia, 2 were Extended Spectrum Betalactamase (ESBL) producers. Other bacterial isolates were sensitive to all drugs.

- *Candida albicans*(66.6%) was the most commonly isolated Candida species from Oral thrush compared to *non-albicans* Candida species(33.3%).

- Among the *non-albicans* Candida species isolated from oral lesions, *C.tropicalis* was predominant(3/6), while *C.dubliniensis*, *C.krusei* and *C.parapsilosis* were isolated one each.

- The antifungal susceptibility testing of the *Candida* species revealed that one *C.albicans* was resistant to Fluconazole and Ketaconazole while another was resistant to Itraconazole. Other *C.albicans* and non-*albicans Candida* were susceptible to all the antifungal drugs tested except *C.krusei* which is intrinsically resistant to Fluconazole.

- Two *Cryptococcus neoformans* were isolated from CSF in patients presenting with meningitis.

- *Strongyloides stercoralis* was identified in one patient with diarrhoea.

- The prevalence of OIs showed an increasing trend with increase in WHO clinical staging.

- Prevalence rate of opportunistic infections showed significant association of increasing trend with decrease in CD4 cell count.

- Prevalence of Candidiasis was more common in patients with CD4 count <200cells/µl and revealed statistically significant association.

- Prevalence of Mycobacterial infection showed increasing trend with decrease in CD4 cell count.

- Mortality was observed in 3 patients included in the study where 2 patients had Tuberculous Meningitis and the other one diagnosed with Cryptococcal meningitis.

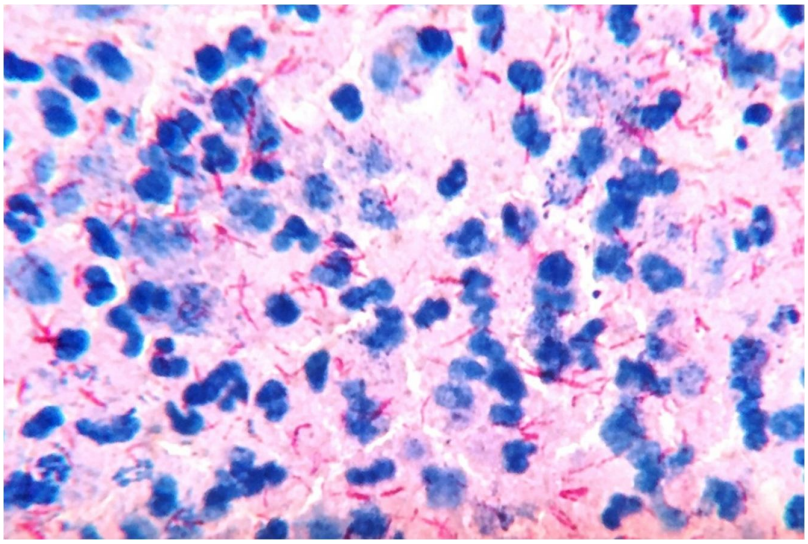
Conclusion

CONCLUSION

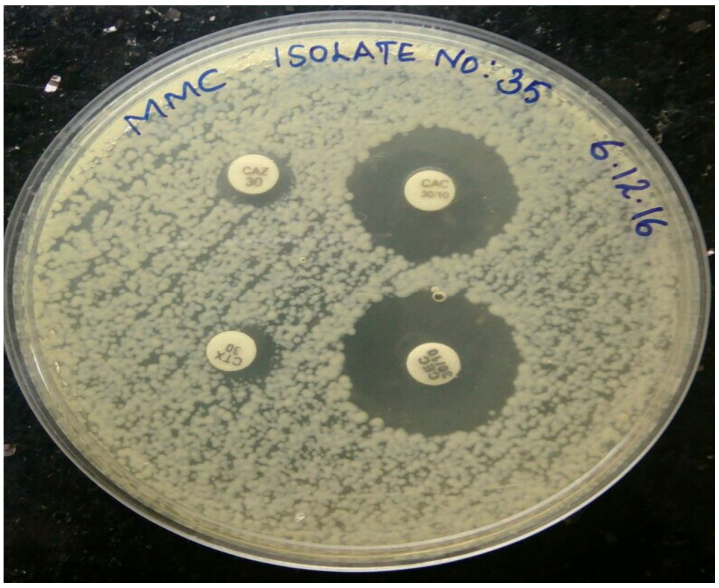
The emergence and pandemic spread of AIDS constitute the greatest challenge to public health in modern time. With the changing scenario of AIDS epidemic, opportunistic infections add to the endemicity of the already existing infections like tuberculosis. The present study reflects that Tuberculosis followed by Candidiasis were the most common OIs seen in PLHA and the risk of OI increases with decrease in CD4 count. Hence this study proves that the spectrum of opportunistic infections among various patient groups varies significantly. Prompt HAART initiation, along with monitoring patients for OIs with decreasing CD4 count, initiating OI prophylaxis, early diagnosis and treatment of OIs will aid in decreasing the morbidity and mortality of the PLHA.

Colour plates

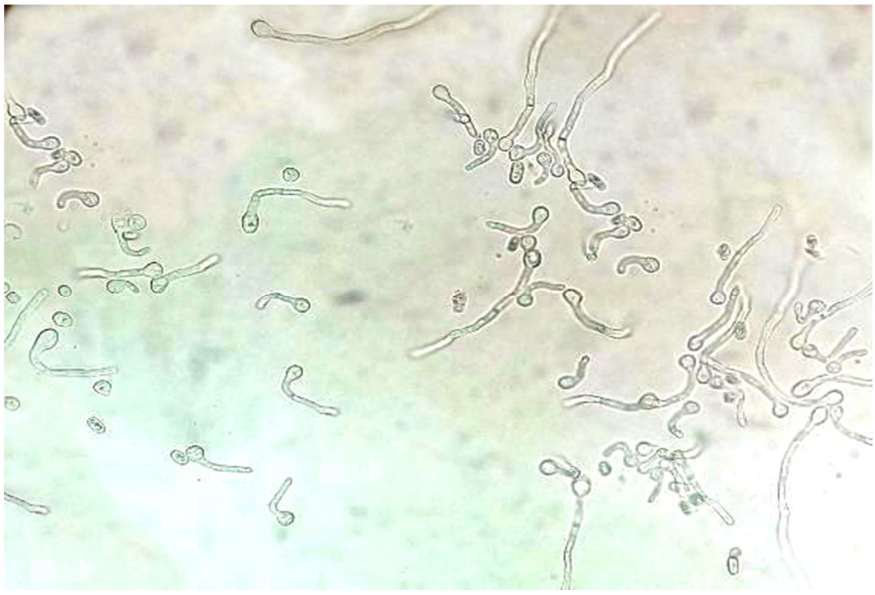
COLOUR PLATES



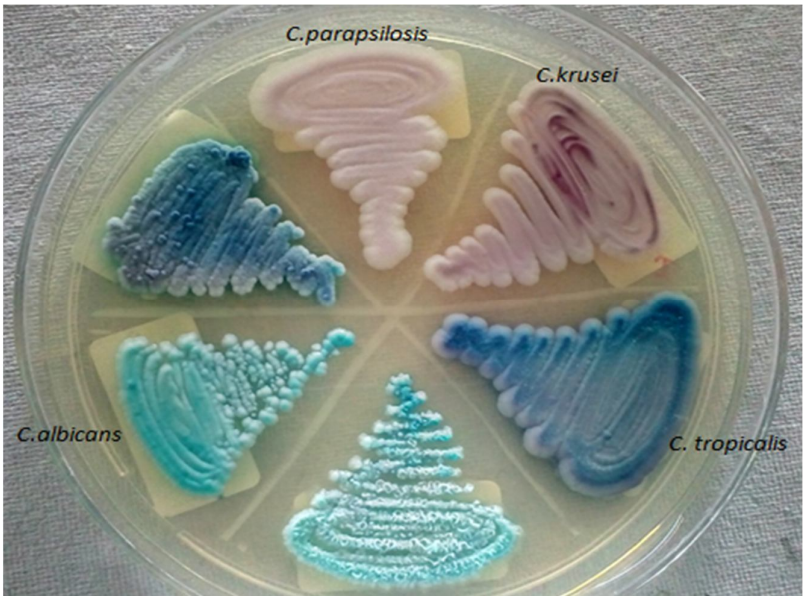
Colour Plate 1: Ziehl-Neelsen staining of Lymph Node aspirate showing Acid Fast Bacilli



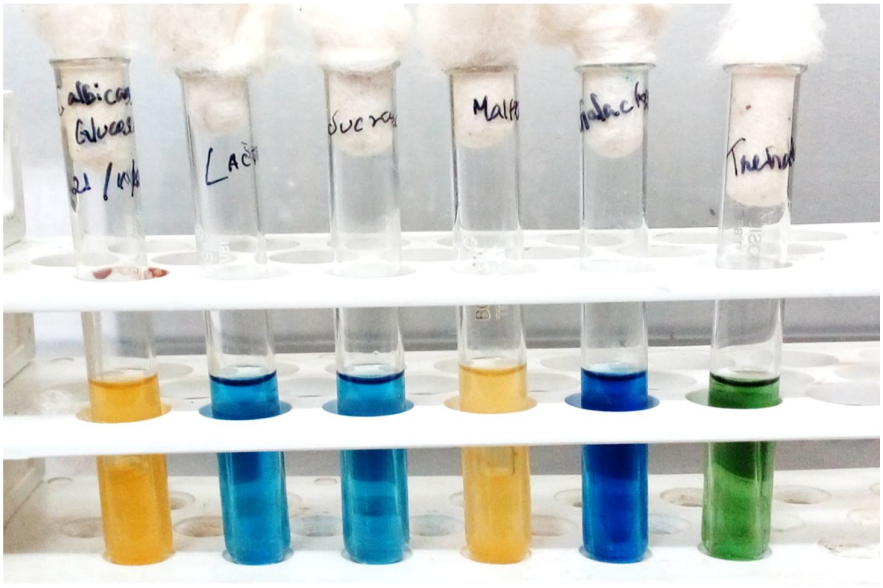
Colour Plate 2: Phenotypic test for the detection of ESBL production by Combined Disc method.



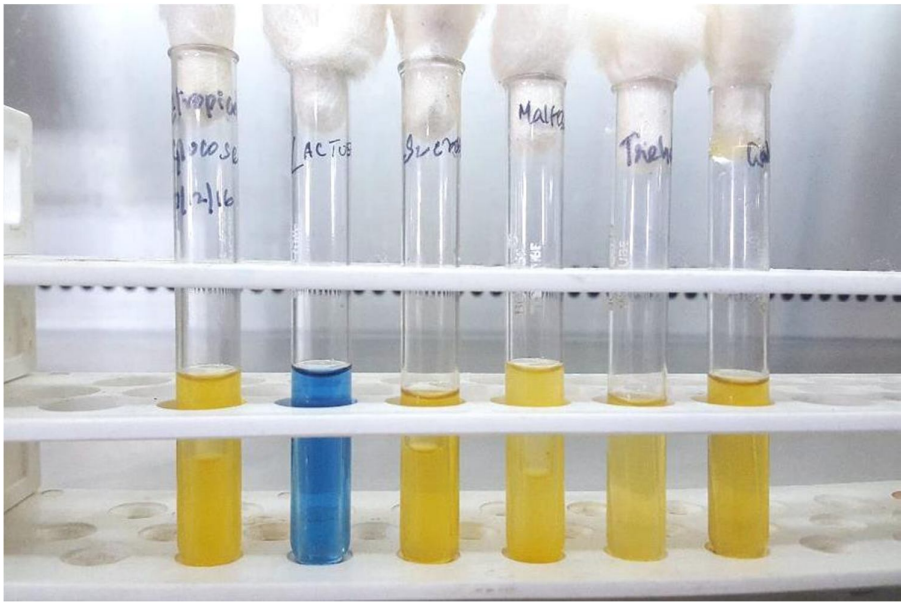
Colour Plate 3: Germ tube production by *C.albicans*



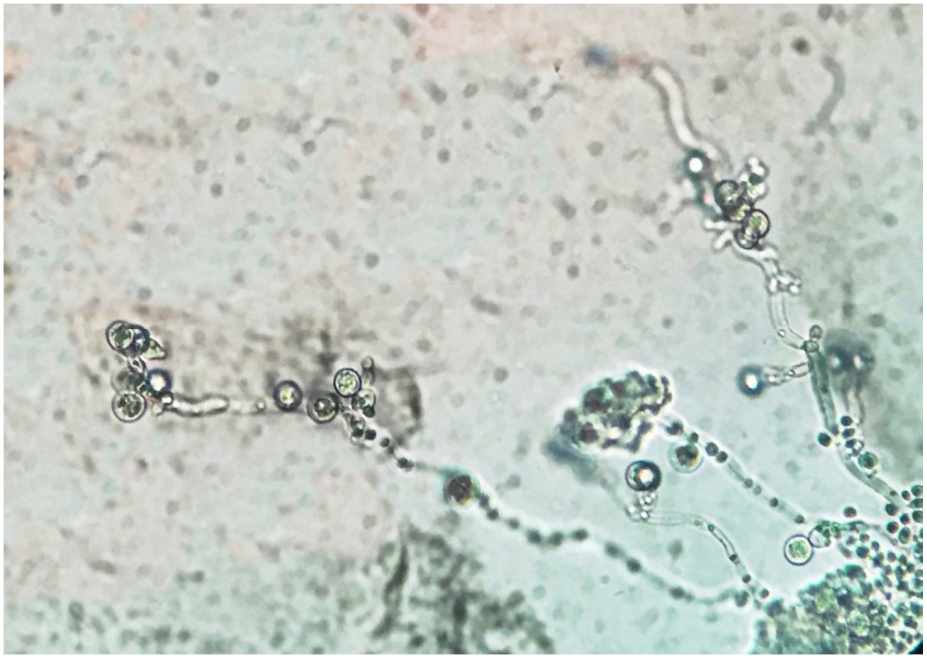
Colour Plate 4: CHROMagar candida



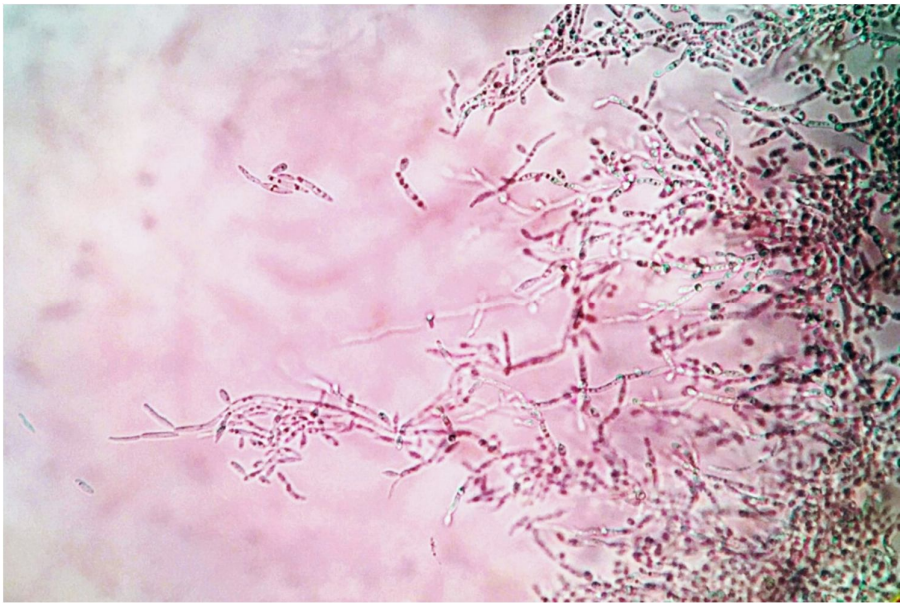
Colour Plate 5 : 2% carbohydrate fermentation test showing reaction for *C.albicans*



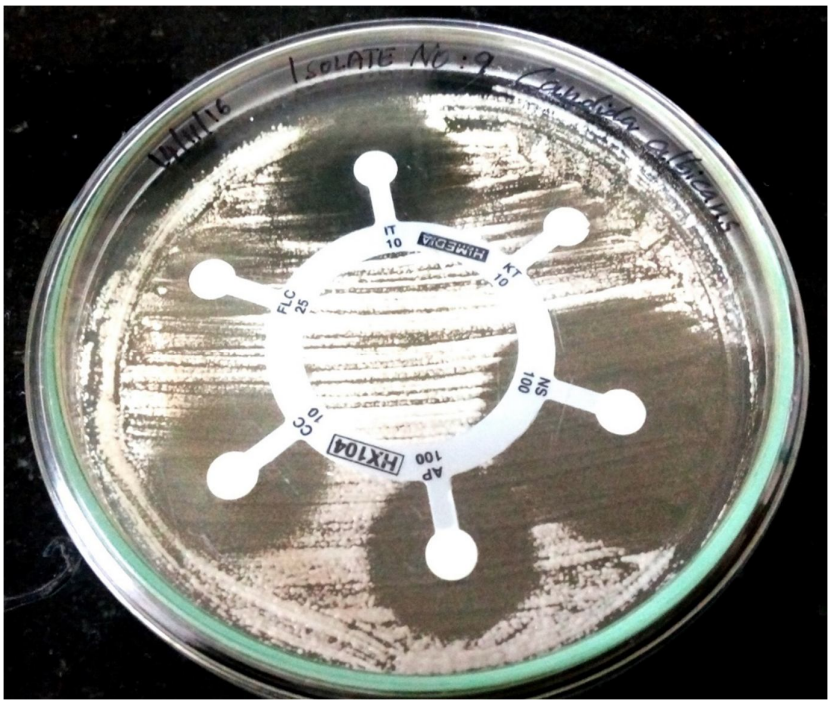
Colour Plate 6: 2% carbohydrate fermentation test showing reaction for *C.tropicalis*



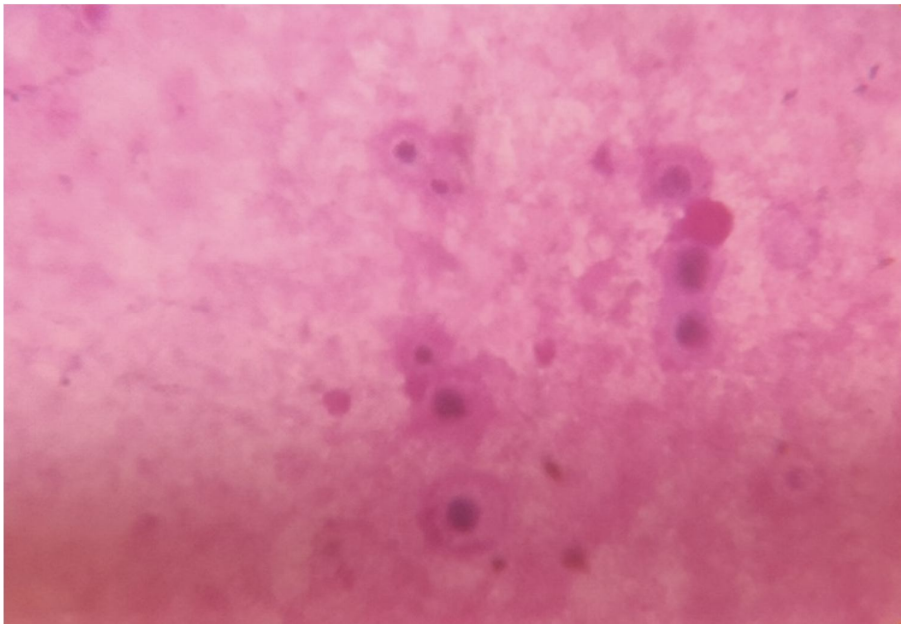
Colour Plates 7: Refractile single terminal Chlamydospore of *C.albicans* on Corn Meal Agar



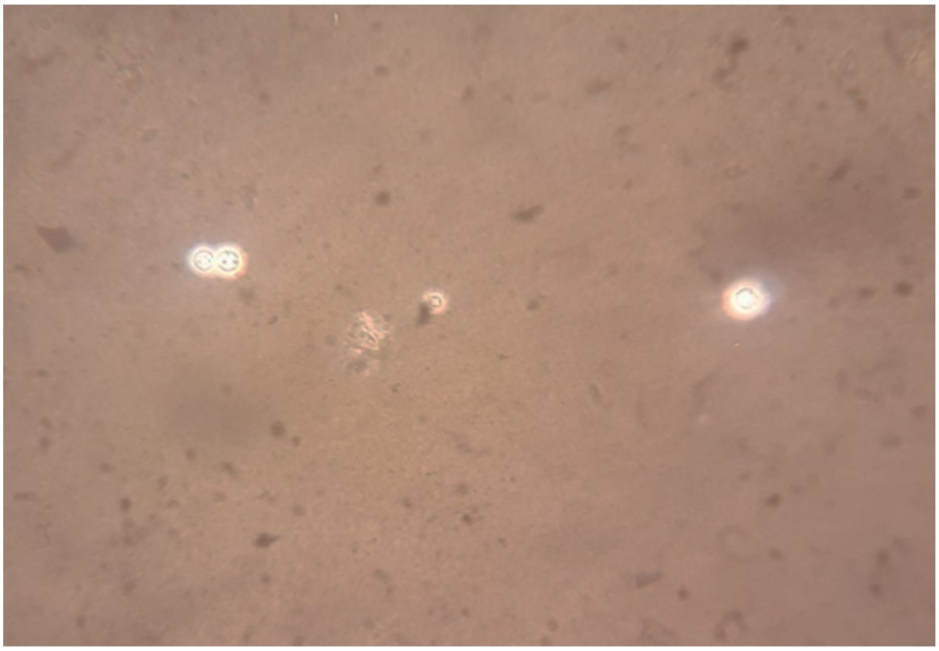
Colour Plates 8: Pseudohyphae with elongated blastoconidia (with cross-match stick appearance) of *C.krusei* on Corn Meal Agar



Colour Plate 9: Antifungal susceptibility testing by Disc Diffusion method



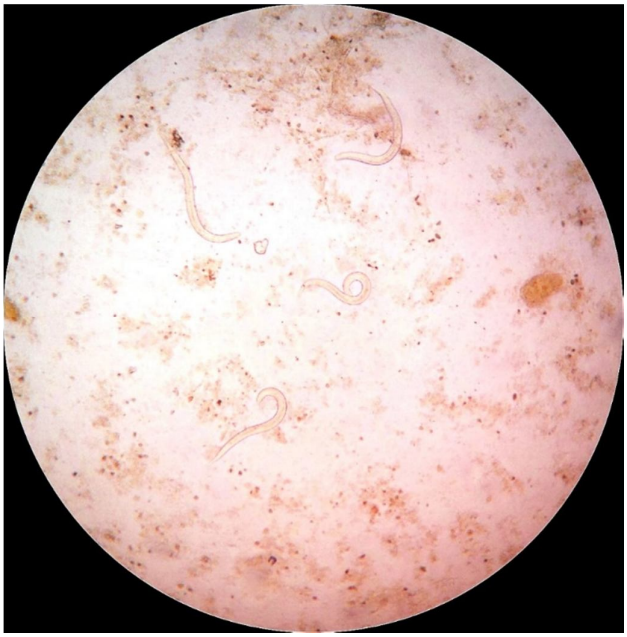
Colour Plate 10: CSF Gram stain of PLHA with meningitis showing capsulated Gram positive round budding yeast cells.



Colour Plate 11: India ink stain of CSF from patient with meningitis showing capsulated round budding yeast cells.

Colour Plate 12: Lateral Flow Assay for Cyptococcal Antigen on CSF with meningitis showing positive test

Colour Plate 13: ***Cryptococcus neoformans*** **showing brown coloured colonies on Bird Seed Agar**



Colour Plate 14: Stool Wet Mount showing Rhabditiform larvae of ***Strongyloides stercoralis***

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ANNEXURE -1

LIST OF ABBREVIATIONS

ADCC	-	Antibody Dependent Cell Mediated cytotoxicity
AFB	-	Acid Fast Bacilli
AIDS	-	Acquired Immuno Deficiency Syndrome
ART	-	Anti-Retroviral Therapy
BHI	-	Brain Heart Infusion
CCR5	-	C-C Chemokine receptor R5
CD	-	Cluster of Differentiation
CDC	-	Centre for Disease Control
CLSI	-	Clinical Laboratory Standards Institute
CSF	-	Cerebrospinal Fluid
CXCR4	-	Co-receptor X4
DNA	-	Deoxy Ribonucleic acid
EDTA	-	Ethylene Diamine Tetra Acetic Acid
ELISA	-	Enzyme Linked Immunosorbent Assay
ESBL	-	Extended Spectrum Betalactamase
FDC	-	Follicular Dentritic Cells
GALT	-	Gut Associated Lymphoid Tissue
GRID	-	Gay-related immune deficiency
HAART	-	Highly Active Anti-retroviral Therapy
HIV	-	Human Immunodeficiency Virus
HTLV	-	Huma T-cell leukemia virus
ICTC	-	Integrated Counselling and Testing Centre
IRIS	-	Immune Reconstitution Inflammatory Syndrome
KOH	-	Potassium hydroxide

LAV	-	Lymphadenopathy-associated Virus
LTR	-	Long Terminal Repeat
LTNP	-	Long Term Non Progressor
NACO	-	National AIDS Control Organization
NALC	-	N-acetyl L-cysteine
NNRTI	-	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	-	Nucleoside Reverse Transcriptase Inhibitor
NTM	-	Non-tuberculous Mycobacteria
OIs	-	Opportunistic Infections
PCP	-	*Pneumocystis jirovecii* Pneumonia
PCR	-	Polymerase Chain Reaction
PI	-	Protease Inhibitor
PLHA	-	People Living with HIV and AIDS
RNA	-	Ribonucleic Acid
RT	-	Reverse Transcriptase
SDA	-	Sabouraud dextrose agar
SPSS	-	Statistical Package for Social Sciences
TB	-	Tuberculosis
UNAIDS	-	The Joint United Nations Programme on HIV and AIDS
WHO	-	World Health Organization

ANNEXURE – II

PROFORMA

- Name : IP/OP NO:
- Age:
- Sex:
- Occupation:
- Address:
- Clinical Diagnosis:

- Presenting complaints

- Personal history

- Past history

- Co-morbid conditions, if any:

- Prior antimicrobial therapy

- CD4 count:

- WHO Clinical stage:

- Microbiological investigation:

- Antibiotic or Antifungal sensitivity pattern:-

ANNEXURE-2

CONSENT FORM

STUDY TITLE : **Prevalence and profile of Bacterial, Fungal And Parasitic Opportunistic infections in People Living With HIV/AIDS**

I……, hereby give consent to participate in the study conducted by Dr.C. Justine Auxilia Irene, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my clinical Specimen (sputum, endotracheal aspirate, bronchial wash, pleural fluid, stool, oral swab, blood, cerebrospinal fluid) for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature/ Thumb impression Of the patient/ relative

Place

Date

Patient Name & Address:

Signature of the investigator:

Signature of guide

ANNEXURE-3

INFORMATION SHEET

STUDY TITLE : **Prevalence of Bacterial, Fungal And Parasitic Opportunistic infections in People Living With HIV/AIDS**

INVESTIGATOR : **Dr.C.Justine Auxilia Irene,**
Post Graduate,
Institute of Microbiology,
Madras Medical College, Chennai - 600003.

GUIDE : **Dr. U.Umadevi,**
Professor of Microbiology,
Institute of Microbiology,
Madras Medical College, Chennai 600 003.

Progressive destruction of immune system by chronic HIV infection leading to progressive fall in the level of CD4 cells (<200/µl to <50 / µl) is known to be responsible for the occurrence of infections by a variety of opportunistic microorganisms. In HIV infection Opportunistic Infections (OIs) account for a considerable proportion of mortality and morbidity . This is also responsible for the recurrent, prolonged, intractable and severe nature of infection in HIV seropositive individuals.

The incidence of OIs depends on the level of immunosuppression and on the endemic prevalence of the pathogen. The organism causing OIs usually cause asymptomatic or mildly symptomatic, self limiting infections in immunocompetent individuals whereas in HIV infected patients, these infections may be severe or even life threatening.

Despite the availability of ART, OIs continue to cause considerable morbidity and mortality because of issues of non-adherence, ART drug resistance and treatment failure. Knowledge of the pattern of opportunistic infections can often guide therapy when resource limitations hamper the exact diagnosis of the etiological agent. HIV/AIDS may not be curable but most of the opportunistic infections can be effectively treated. Prophylaxis against some of these infections will not only prolong the life of an HIV infected individual but also improve the quality of life

.

The type of pathogens responsible for OIs varies from country to country and even from region to region within the same country . Therefore it is important to know the relative frequencies of specific OIs in different parts of the country for appropriate management strategies. Considering this fact, the present study is to be conducted to determine the prevalence of bacterial, parasitic, fungal infections in HIV seropositive patients, to assess the pattern of sensitivity of the isolates

This study is entirely voluntary and patient can withdraw any time from this study. Extra cost will not be incurred to the patients in this study. Confidentiality of the patient's detail will be maintained at all level. Any queries regarding this study will be willingly clarified. Results of the study will be published. In case of any doubts please contact Dr.C.Justine Auxilia Irene

ANNEXURE IV - MASTER CHART

S.No	IP. No	AGE	SEX	CLINICAL PRESENTATION	WHO STAGE	CD4 COUNT	SAMPLE	ORGANISM	RIF	AK	OF	CIP	COTRI	CTX	CAZ	PT	TET	IMP	AMP B	NYS	FLU	CLO TRI	KETA	ITRA
1	61511	29	F	Headache	IV	210	CSF	*Cryptococcus neoformans*																
2	63757	46	M	Fever	IV	336	Blood	NIL																
3	81146	50	M	Fever, cough	IV	352	Sputum	NIL																
4	79954	55	M	Headache, altered sensorium	IV	432	CSF	Mycobacteria	S															
5	84583	50	F	Cough with expectoration	IV	512	Sputum	NIL																
6	85122	45	F	Acute diarrhea	IV	95	Stool	NIL																
7	68095	38	M	Fever, cough	IV	92	Sputum	Mycobacteria	S															
8	67293	50	M	Fever	IV	223	Blood	NIL																
9	68926	30	M	Chronic diarrhea	III	133	Stool	NIL																
10	79780	37	M	Chronic diarrhea	IV	41	Stool	*Strongyloides stercoralis*																
11	90027	29	F	Cough with expectoration	II	536	Sputum	NIL																
12	90197	50	F	Cough with expectoration	III	452	Sputum	NIL																
13	94878	35	F	Fever, cough with expectoration	II	258	Sputum	*K.pneumoniae*		S	S	S	S	S			S	S						
14	95398	45	M	Breathlessness, cough	IV	342	Sputum	NIL																
15	91427	38	F	Cough with expectoration	III	190	Sputum	NIL																
16	95394	42	M	Acute diarrhea	III	236	Stool	NIL																
17	94808	52	M	Fever, cold abscess	IV	410	Lymph node aspirate	Mycobacteria	S															
18	98188	40	M	Fever, cough with expectoration	IV	77	Sputum	NIL																
19	96963	40	M	Cough with expectoration	III	259	Sputum	NIL																
20	99169	40	M	Oral thrush	IV	35	oral swab	*C.albicans*											S	S	S	S	S	S
21	92236	28	M	Fever, Headache	IV	34	CSF	Mycobacteria	S															
22	105729	45	M	Fever, cough with expectoration	III	459	Sputum	*P.aeruginosa*		S	S	S			S	S		S						
23	105218	35	F	Cough with expectoration	III	197	Sputum	*K.pneumoniae*		S	S	R	R	S			S	S						
24	105499	43	M	Fever, breathlessness	IV	34	Sputum	Mycobacteria	S															
25	102027	37	M	Fever, cough	III	539	Sputum	NIL																
26	107680	48	M	Cough with expectoration	III	251	Sputum	NIL																
27	115140	26	M	Cough	II	587	Sputum	NIL																
28	10302	38	M	Breathlessness, cough	II	544	Sputum	NIL																
29	107928	45	M	Fever, breathlessness	IV	34	Sputum	*P.jirovecii*																
30	11070	39	M	Fever, altered sensorium	IV	133	CSF	NIL																
31	10962	45	F	Oral thrush	III	328	oral swab	*C.albicans*											S	S	S	S	S	S
32	11959	32	F	Fever, cough with expectoration	IV	86	Sputum	NIL																
33	10872	50	F	Fever, breathlessness	III	445	Sputum	NIL																
34	18479	40	M	Cough with expectoration	III	630	Sputum	NIL																
35	18462	40	M	Cough with expectoration	IV	450	Sputum	NIL																
36	17350	50	M	Chest pain, breathlessness	IV	344	Sputum	NIL																
37	14212	36	M	Cough with expectoration	IV	137	Sputum	NIL																
38	13429	29	M	Oral thrush	IV	119	oral swab	*C.tropicalis*											S	S	S	S	S	S
39	15928	40	M	Cough	IV	47	Sputum	NIL																
40	13804	36	F	Breathlessness, cough	IV	123	Sputum	NIL																
41	17552	28	F	Fever, cough with expectoration, oral thrush	IV	155	Sputum, Oral swal	Mycobacteria, *C.tropicalis*											S	S	S	S	S	S
42	21238	43	M	Fever, breathlessness	IV	126	Sputum	NIL																
43	22469	35	F	Cough with expectoration	IV	512	Sputum	NIL																
44	22178	36	F	Cough with expectoration	IV	450	Sputum	NIL																
45	110501	32	M	Fever, chest pain	IV	321	Sputum	NIL																
46	17592	50	M	Breathlessness, cough	III	161	Sputum	Mycobacteria	S															
47	23746	40	F	Cough with expectoration	IV	123	Sputum	NIL																
48	23406	55	M	Fever, cough	IV	542	Sputum	NIL																
49	29913	40	M	Fever, breathlessness	IV	57	Sputum	NIL																
50	21633	34	F	Breathlessness, cough	III	228	Sputum	NIL																

S.No	IP. No	AGE	SEX	CLINICAL PRESENTATION	WHO STAGE	CD4 COUNT	SAMPLE	ORGANISM	RIF	AK	OF	CIP	COTRI	CTX	CAZ	PT	TET	IMP	AMP B	NYS	FLU	CLO TRI	KETA	ITRA
51	23947	42	F	Fever, hemoptysis	III	354	Sputum	Mycobacteria	S															
52	26087	46	M	Fever, headache, cough with expectoration	IV	51	CSF,Sputum	Mycobacteria, *K.pneumoniae*	S	S	S	S	S	R			S	S						
53	28782	22	F	Fever, cough	IV	423	Sputum	NIL																
54	30354	45	F	Breathlessness, chest pain	IV	145	Sputum	NIL																
55	28521	40	M	Fever, cough with expectoration	III	211	Sputum	Mycobacteria																
56	29116	63	M	Fever, loss of appetite	IV	127	Sputum	NIL																
57	5727	56	F	Loss of appetite, breathlessness	III	125	Sputum	NIL																
58	5385	34	F	Fever, fatigue, cough with expectoration	II	154	Sputum	NIL																
59	5676	40	F	Cough with expectoration	IV	58	Sputum	Mycobacteria	S															
60	5872	46	F	Breathlessness, cough	IV	340	Sputum	NIL																
61	5378	35	M	Oral thrush	IV	143	Oral swab	C.albicans											S	S	S	S	S	S
62	16147	70	M	Diarrhea	IV	180	Stool	NIL																
63	7722	44	F	Chest pain, loss of appetite	IV	124	Sputum	NIL																
64	11223	34	F	Hemoptysis	IV	228	Sputum	Mycobacteria	S															
65	6022	54	M	Fever, cough	IV	33	Sputum	Mycobacteria	S															
66	3317	47	M	Cough with expectoration	IV	146	Sputum	Mycobacteria																
67	613	36	M	Fever, breathlessness	IV	156	Pleural Fluid	NIL																
68	13102	30	M	Breathlessness, cough	II	727	Sputum	NIL																
69	10563	36	M	Fever, cough	III	185	Sputum	NIL																
70	13123	27	F	Fever, cough with expectoration	IV	46	Sputum	Mycobacteria	S															
71	833	54	M	Easy fatigue, fever, breathlessness	III	321	Sputum	NIL																
72	117196	44	M	Cough with expectoration	III	94	Sputum	Mycobacteria	S															
73	38700	40	F	Breathlessness, cough with expectoration	III	81	Sputum	Mycobacteria																
74	55560	43	M	Fever, cough with expectoration	II	623	Sputum	Mycobacteria																
75	45321	55	M	Headache, neck stiffness	IV	32	CSF	*Cryptococcus neoformans*																
76	69432	28	M	Oral thrush	III	65	Oral swab	NIL																
77	43786	51	F	Fever, cough	III	420	Sputum	NIL																
78	47734	24	F	Cough with expectoration	IV	124	Sputum	Mycobacteria																
79	46655	32	M	Acute diarrhea	IV	134	Stool	NIL																
80	30449	42	M	Chest pain, breathlessness	II	643	Sputum	NIL																
81	48296	53	M	Oral thrush	IV	128	Oral swab	*C.albicans*											S	S	S	S	S	S
82	47812	44	F	Oral thrush	IV	52	Oral swab	*C.krusei*											S	S	R	S	S	S
83	59883	32	M	fever,myalgia,cough	III	256	Sputum	NIL																
84	50037	41	M	fever, easy fatigue	III	154	Blood	NIL																
85	45821	53	M	fever,loss of appetite	IV	223	Blood	NIL																
86	59344	35	F	Acute diarrhea	IV	54	Stool	NIL																
87	48936	43	F	Oral thrush	IV	64	Oral swab	*C.tropicalis*											S	S	S	S	S	S
88	52085	34	M	fever,altered sensorium	III	143	CSF	NIL																
89	52093	46	M	fever	III	526	Blood	NIL																
90	58287	40	M	Cough with expectoration	IV	114	Sputum	NIL																
91	59464	48	M	Cold abscess, loss of weight	III	476	Lymph node aspirate	NIL																
92	57634	36	M	Fever, cough	III	231	Sputum	NIL																
93	50954	25	F	Oral thrush	IV	134	Oral swab	NIL																
94	64785	39	M	Oral thrush	III	154	Oral swab	*C.albicans*											S	S	S	S	S	R
95	65687	33	M	Loss of weight, Lymphadenopathy	IV	32	Lymph node aspirate	NIL																
96	54678	41	F	Cough with expectoration	III	119	Sputum	Mycobacteria																
97	67435	47	F	Fever, breathlessness	IV	235	Sputum	NIL																
98	68975	38	M	Fever, loss of weight	IV	227	Blood	NIL																
99	62484	22	M	Chronic diarrhea	IV	162	Stool	NIL																

S.No	IP. No	AGE	SEX	CLINICAL PRESENTATION	WHO STAGE	CD4 COUNT	SAMPLE	ORGANISM	RIF	AK	OF	CIP	COTRI	CTX	CAZ	PT	TET	IMP	AMP B	NYS	FLU	CLO TRI	KETA	ITRA
100	78425	46	M	Breathlessness, cough	II	546	Sputum	NIL																
101	74563	31	M	Diarrhea	IV	176	Stool	NIL																
102	75683	24	M	Oral thrush	III	132	Oral Swab	*C.albicans*											S	S	S	S	S	S
103	78645	43	M	Fever, seizures	IV	356	CSF	NIL																
104	65274	36	F	Cough, hemoptysis	IV	49	Sputum	Mycobacteria	S															
105	66843	49	F	Fever, lymphadenopathy	III	565	Lymph node aspirate	NIL																
106	74865	32	F	Oral thrush	IV	176	Oral swab	*C.albicans*											S	S	S	S	S	S
107	72497	36	M	Cough with expectoration	IV	87	Sputum	Mycobacteria	S															
108	74683	49	M	Fever, chest pain	III	235	Sputum	NIL																
109	74795	48	M	Fever, cough	II	519	Sputum	NIL																
110	81231	34	M	Oral thrush	III	132	Oral swab	*C.dubliniensis*											S	S	S	S	S	S
111	87644	27	M	Fever, loss of weight	II	132	Blood	NIL																
112	83530	47	M	Fever	IV	385	Blood	NIL																
113	86073	38	F	Fever, cough	III	348	Sputum	NIL																
114	89579	46	F	Fever, cough	II	584	Sputum	NIL																
115	64849	36	M	Oral thrush	IV	210	Oral swab	*C.albicans*											S	S	S	S	S	S
116	86746	43	M	Cough , breathlessness	IV	38	Sputum	NIL																
117	43795	44	M	Cough with expectoration	IV	234	Sputum	NIL																
118	54738	37	F	Chronic diarrhea	II	198	Stool	NIL																
119	57838	34	M	Cough with expectoration	IV	176	Sputum	*K.pneumoniae*		S	S	S	S	S			S	S						
120	57857	35	M	Fever, cough	III	132	Sputum	NIL																
121	83526	35	M	Diarrhea	IV	76	Stool	NIL																
122	84783	37	M	Cough, chest pain	IV	75	Sputum	*Mycobacteria*	S															
123	67583	32	M	Oral thrush	IV	148	Oral swab	C.albicans											S	S	R	S	R	S
124	89647	36	M	Acute diarrhea	III	658	Stool	NIL																
125	75953	45	M	Oral thrush	IV	65	Oral swab	*C.parapsilosis*											S	S	S	S	S	S
126	23536	31	F	Fever, cough	IV	154	Sputum	*Mycobacteria*																
127	45467	45	M	Diarrhea	IV	78	Stool	NIL																
128	54367	43	F	Diarrhea	III	564	Stool	NIL																
129	75467	37	F	Fever, breathlessness	IV	452	Sputum	NIL																
130	26286	29	M	Oral thrush	IV	196	Oral swab	*C.albicans*											S	S	S	S	S	S
131	67697	43	M	Fever	IV	123	Blood	NIL																
132	65478	36	F	Chronic diarrhea	IV	265	Stool	NIL																
133	21456	24	F	Fever, cough	II	243	Sputum	NIL																
134	76464	34	M	Fever, cough	II	536	Sputum	NIL																
135	87497	36	F	Breathlessness, cough with expectoration	II	678	Sputum	NIL																
136	64869	38	M	Cough with expectoration	IV	413	Sputum	NIL																
137	75496	27	M	Oral thrush	IV	122	Oral swab	*C.albicans*											S	S	S	S	S	S
138	76483	45	M	Diarrhea	IV	84	Stool	NIL																
139	74686	38	F	Cough with expectoration	III	143	Sputum	Mycobacteria	S															
140	86486	34	F	Oral thrush	III	163	Oral swab	*C.albicans*											S	S	S	S	S	S

ANNEXURE-5

LEGENDS OF MASTER CHART

AK	-	Amikacin
AMP B	-	Amphotericin B
CAZ	-	Ceftazidime
CLOTRI	-	Clotrimazole
COTRI	-	Cotrimoxazole
CTX	-	Cefotaxime
FLU	-	Fluconazole
IMP	-	Imipenem
ITRA	-	Itraconazole
KETA	-	Ketaconazole
NYS	-	Nystatin
OF	-	Ofloxacin
PT	-	Piperacillin-Tazobactam
R	-	Resistant
RIF	-	Rifampicin
S	-	Susceptible
TET	-	Tetracycline

ANNEXURE-6

IINSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.C.Justine Auxilia Irene
Post Graduate in M.D.Microbiology
Institute of Microbiology
Madras Medical College
Chennai 600 003

Dear Dr.C.Justine Auxilia Irene,

The Institutional Ethics Committee has considered your request and approved your study titled **" PREVALENCE AND PROFILE OF BACTERIAL, FUNGAL AND PARASITIC OPPORTUNISTIC INFECTIONS IN PEOPLE LIVING WITH HIV/AIDS " NO. 17062016.**

The following members of Ethics Committee were present in the meeting hold on **07.06.2016** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr.Isaac Christian Moses,MD.Ph.D.Dean(FAC)MMC,Ch-3	:Deputy Chairperson
3.Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	:MemberSecretary
4.Prof.B.Vasanthi,MD., Prof.of Pharmacology.,MMC,Ch-3	: Member
5.Prof.P.Raghumani,MS, Prof. of Surgery,RGGGH,Ch-3	: Member
6.Prof.Baby Vasumathi, Director, Inst. of O&G,Ch-8	: Member
7.Prof.K.Ramadevi,MD, Director,Inst.of Bio-Chem,MMC,Ch-3	: Member
8.Prof.M.Saraswathi,MD.,Director, Inst.of Path,MMC,Ch-3	: Member
9.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
10.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
11.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

29/6/16

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003