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Review Article

Microbiology Section

Pneumocystis jirovecii Pneumonia: A Revisit to the Old Malady

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ABSTRACT

Pneumocystis jirovecii Pneumonia (PJP) was previously called as *Pneumocystis carinii* Pneumonia (PCP), it is one of the most common opportunistic fungal infection in immuno-compromised individuals. Patients may present clinical symptoms such as fever, non-productive cough, chills, weight loss, dyspnoea, shortness of breath and respiratory failures in severe cases. Clinical samples such as Sputum, Induced sputum, Bronchio Alveolar Lavage fluid (BAL), Pleural fluid and Lung tissue are used for identification of *Pneumocystis* infection. Diagnosis of *Pneumocystis jirovecii* is done by staining, serology, in-vitro cultivation and molecular methods. Different types of Polymerase Chain Reaction (PCR) assays can be used for different gene targets for detection of *Pneumocystis*. PCR assays are most sensitive and specific methods which can be useful for detection of *P. jirovecii* from infected individuals, recurrent PJP patients. Treatment of PJP infection is based on the severity of the illness and diagnosis. Evidence based guidelines should be followed for giving proper treatment. If treatment is not given, the patient will die with PJP infection. Trimethoprim/Sulfamethoxazole (TMP-SMZ) is given as an early treatment prophylaxis in patients with PJP. If side effects are severe with TMP-SMZ, other drugs include Pentamidine, Trimetrexate, Atovaquone, clindamycin and primaquine combined with leucovorin, dapsone and Caspofungin combined with clindamycin can be given as alternative drug regimen to the patients with PJP infection. Wide spread and long term usage of PJP prophylaxis can lead to rising new cases of dihydropteroate synthetase (DHPS) mutant strains. Extensive utilisation of TMP-SMZ and dapsone in Human Immunodeficiency Virus (HIV) and other immuno-compromised individuals can lead to sulfa (sulfonamide or sulfone) drug resistance. Evaluation of drug resistance is complicated in HIV patients and finally further advance research is required for strategies development as well as to stop the further increase of resistant strains. New diagnostic methods and usage of non-invasive respiratory specimens such as oral washes for diagnosis of PJP should be expanded. PCR is the most sensitive method compared to other conventional and serological methods. Using PCR and Sequence analysis can be helpful for estimation of prevalence of disease as well as epidemiological purposes. There is an urgent need to develop newer drugs and vaccines to eradicate the PJP disease burden. This review of literature gives recent information on *PJP* by highlighting epidemiology, taxonomy, pathophysiology, mortality, present situation, recent diagnostic methods, recommended immuno-prophylaxis and recent advances.

Keywords: Andhra Pradesh, Human immunodeficiency virus, Immuno-compromised

INTRODUCTION

PJP was previously called as PCP, it is one of the most common opportunistic fungal infection in immuno-compromised conditions such as haematological malignancy, congenital immunodeficiency, organ transplantation, immunosuppressive therapy, under medication and predominantly in HIV [1]. Patients presenting with clinical symptoms are fever, non productive cough, chills, weight loss, dyspnoea, shortness of breath and respiratory failures may also occur in severe cases [2]. Person to person PJP transmission typically takes place via air borne route. If healthy individuals are infected, asymptomatic lung colonisation can occur in these people and act as asymptomatic carriers. These people can spread infection to immuno-compromised individuals; it may cause severe respiratory infection particularly in AIDS patients (AIDS associated Pneumonia). Still, *Pneumocystis* is a major opportunistic atypical fungus causing infection to immuno-compromised individuals [3]. Occurrence of severe *Pneumocystis* infection may lead to morbidity and mortality in healthy as well as immuno-compromised individuals. But availability of specific chemoprophylaxis, effective combination Highly Active Anti-Retro Viral Therapy (HAART) decreases opportunistic infections in HIV patients. Extensive prophylaxis can diminish the disease burden in this group of population [4-6].

MICROBIOLOGY

Nomenclature and Controversy on Taxonomy of *Pneumocystis jirovecii*

In the year 1906, Carlos Chagas identified *Pneumocystis* for the first time and placed them under the group of protozoan. Again in 1988, they were reclassified and considered as an ascomycetous group of fungus [6-8]. Life cycle of *Pneumocystis* have similar characters of both protozoan and fungus. Researchers conducted phylogenetic analysis of small subunit ribosomal RNAs and compared lineages of plant, animal, fungi and protozoa [9,10]. Finally, researchers concluded that *Pneumocystis* comes under the fungal group based on the characters of cell wall composition, enzyme structure and sequencing genes. Species of *Pneumocystis* are *Pneumocystis carinii* and *Pneumocystis jirovecii* which infect rats and humans respectively.

The scientist Otto jirovec identified the organism in humans and gave the name *Pneumocystis jirovecii* instead of *P. carinii* [7-10].

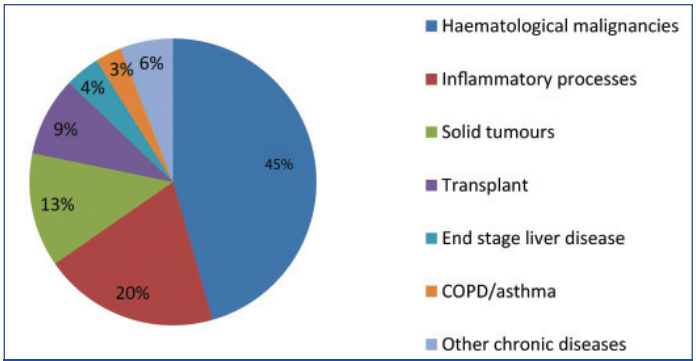
EPIDEMIOLOGY

First Case Report of PJP

First clinical case of *Pneumocystis* was recognised during World War II in Europe among premature and malnourished children. Later, cases were reported in children in Iranian orphanages. In 1981, *Pneumocystis* infection was reported in homosexuals and intravenous drug users [11,12].

Risk Factors of PJP

Major risk factors for acquiring disease of PJP include HIV, solid organ transplantation, malignancy, congenital immuno-deficiency and immuno-suppressive therapy. In non-infected HIV individuals, immuno-suppressive therapy with glucocorticoids was the major risk factor for acquiring PJP [3,6,13,14] [Table/Fig-1].



[Table/Fig-1]: *Pneumocystis* infection varies based on the diagnosis and disease condition.

HIV Epidemic Related to PJP

HIV outbreak occurred in 1980; studies focused on illness of disease and found PJP as the important risk factor. Thereafter, PJP turned from rare disease to common pneumonia. Almost 75% of the HIV patients acquired *Pneumocystis* infection during their life time. Disease rate decreased from 1989, due to beginning of prophylaxis and anti-retroviral therapy. The rate of infection decreased slowly in developed countries and also in United States. Recurrence of PJP can occur more in patients with HIV infection than in non-HIV patients. Still, it is a common infection in immuno-compromised individuals especially in HIV patients and also other conditions that weaken the immune system [4,15].

In United States and Canada, PCP was the common most opportunistic infection for the period of 2008-2010 [16]. In US, no national surveillance programme was conducted on PJP. That's the reason why exact number of cases was not reported from U.S. [17]. Cohort study was conducted among 8500 HIV infected individuals in Europe. This study reported decrease in incidence of PCP before cART 4.9 cases per 100 person-years and after cART 0.3 cases per 100 person-years [18]. During 1980s, 0-11% of *Pneumocystis* infections were reported in AIDS patients. Trend of *Pneumocystis* infection occurred in AIDS patients were raised in Africa. Higher rate of infection were reported in the year of 1995 & 2002 in Zimbabwe 33% and Zambia 22% [19-21].

Non-HIV Epidemic Related to PJP

Pneumocystis infection occurred in non-HIV individuals who are Immuno-suppressed.

Indian Perspective: Singh YN et al., first reported three cases of *Pneumocystis* infection with AIDS disease in 1993 [Table/Fig-2] [22-35].

In Indian tertiary care set-up, very less number of studies were reported on nosocomial PJP. In 2014, PJP outbreak was reported at a tertiary care hospital [30].

Mortality

Kumarasamy N et al., reported, 22% of deaths in AIDS patients with infection of *Pneumocystis* in the year of 1996 to 2008 in Chennai (South India) [36]. A study from Mumbai, reported 15.8% deaths occurred in HIV patients, in the year of 2000-2003 [37]. Rajagopalan N et al., reported 2% of death cases in Karnataka, in AIDS patients with *Pneumocystis* (2004-2006) [26]. Deodhar D et al., reported 46.1% of death cases in HIV group of patients and 34.6% in non-HIV group patients in the year of 2009-2014 [32]. Jeswani J et al., reported 4 deaths among 15 cases of renal transplant patients in Jaipur in the year of 2013- 2018 [33].

Present Situation

Most of the studies were conducted broadly on opportunistic infections in HIV disease. Very few studies are available on *Pneumocystis jirovecii*; their detection, genotypes, mutations and drug resistance from Andhra Pradesh and India. Future research will be needed on PJP infection to overcome disease burden.

PATHOPHYSIOLOGY

Pneumocystis jirovecii is a main pathogen causing pneumonia in people with immuno-deficiency state. Basic mechanism of pathogenesis causing pneumonia is not completely understood. *Pneumocystis* reaches the alveoli, first interact with cells and other components. The primary binding is with type 1 alveolar epithelium, fungus transition occurs from trophic form to cystic form. This binding does not damage the alveoli but awake the host inflammatory response. Hypoxia and impaired gas exchange leads to respiratory failure. *Pneumocystis* is an alveolar pathogen but sometimes in immuno-compromised individuals, it acts as a disseminated form. Extra pulmonary manifestations like hepatosplenomegaly, thyroid, ear, ocular and skin lesion were shown by *Pneumocystis* infected immuno-compromised patients [38].

TRANSMISSION

PJP is a communicable disease. Person to person transmission occur through air borne route. If healthy people acquire infection of *Pneumocystis*, organism will be found in their lungs but does not show any signs and symptoms. These people can act as carriers and infection transmits to other people who are immuno-compromised or have lower immunity condition. Majority of the infections and outbreaks were reported in hospital settings. Recurrent pneumonia

Author	Year published	State	PJP
Singh YN et al., [22]	1993	Delhi	First reported three cases of *Pneumocystis* infection with AIDS disease.
Mirdha BR et al., [23]	2000	New Delhi	Reported 4 cases of *Pneumocystis* in 53 AIDS patients.
Merchant RH et al., [24]	2001	Mumbai, Maharashtra	Reported 3.88% of infection in under the age of 5 years.
Kumarasamy N et al., [25]	2005	Chennai, Tamil Nadu	Reported 0.7 to 7% cases in India.
Rajagopalan N et al., [26]	2009	Bangalore, Karnataka	Reported 5% cases.
Shahapur PR et al., [27]	2014	Bijapur, Karnataka	Reported 1.81% cases.
Chawla K et al., [28]	2011	Manipal, Karnataka	Reported 20% *Pneumocystis* cases in HIV patients.
Ramesh K et al., [29]	2015	Bellary, Karnataka	Reported 16% of *Pneumocystis* cases with AIDS.
Jairam A et al., [30]	2014	Delhi, India	Reported outbreak of PJP infection in renal transplant recipients (RTRs).
Kaur R et al., [31]	2016	New Delhi, India	Reported 27.2% of cases.
Deodhar D et al., [32]	2018	Ludhiana, Punjab.	Reported 30.9% of cases.
Jeswani J et al., [33]	2018	Jaipur, Rajasthan	Reported *Pneumocystis* infection in non-HIV group individuals, included renal transplant patients, malignancies, chronic lung disease and connective tissue disorder.
Shilpa et al., [34]	2018	Raichur, Karnataka	Reported 16% of cases.
Singh Y et al., [35]	2019	New Delhi, India	Reported 22.4% cases of *Pneumocystis* in HIV patients.

[Table/Fig-2]: Various PJP reports in India for past 19 years [22-35].

occurred in same persons in different episodes by involvement of genotype. PJP is easily spread in people who are defective of both cellular and humoral immunity [4].

CLINICAL FEATURES

Non-specific physical findings were observed in patients with PJP. Common symptoms are fever, cough, difficulty breathing, chest pain, chills and fatigue. Mild fever was observed in people with HIV infection after several weeks of PJP infection. Almost 7% of the people are asymptomatic. Pulmonary auscultation is common; when abnormal, the most common finding is inspiratory crackles. Extra pulmonary manifestations like thyroiditis, retinitis, bone lesions, pneumocystosis of brain, liver, spleen and kidney were not common in PJP patients but these are common among patient group including advanced AIDS condition, extremely immuno-compromised and treatment taking with aerosolized Pentamidine [39].

DIFFERENTIAL DIAGNOSIS

Differential diagnosis facilitates proper testing to rule out possibilities and prove an absolute diagnosis. Pulmonary complications are developed with atypical presentation and PJP cannot be ruled out. A confident diagnosis requires a grouping of clinical, radiological, and laboratory investigations for giving proper treatment.

Tuberculosis: Opportunistic bacterial and fungal diseases include tuberculosis and PJP may cause illness in patients with HIV [40].

Acute Respiratory Distress Syndrome (ARDS): HIV patients with PJP are associated with a high death rate, which increases significantly with the need for mechanical ventilation after presenting with respiratory distress and severe hypoxemia [41].

***Mycoplasma* infections:** In Pneumonia cases, presence of *P. jirovecii* and *M. pneumoniae* and in the absence of definitive diagnoses, monitoring the treatment response is vital mainly when first line antibiotic predilection is β-lactams or Cephalosporins [42,43].

Pulmonary embolism: Symptoms presented almost same as that of PJP. One of the case reports concluded the final diagnosis with chest radiograph and CT scan [44].

Mycobacterium avium Complex (MAC) Infection [40]. A Case report concluded PJP can rarely occur in patients with HIV as a consequence of immuno-suppression and more frequently presents as extra pulmonary manifestations [45].

Viral pneumonia: Cyto Megalo Virus (CMV) is the most common viral infection in AIDS patients. AIDS-related lymphoma and unclear interstitial pneumonia may become impersonator of PJP. In those cases, serum β-D-glucan examination and PCR for *Pneumocystis* should be valuable for the differential diagnosis [46].

Complications

ARDS, Lymphadenopathy, Pancytopaenia, Respiratory failure [47].

DIAGNOSIS

Multiple factors play a role in diagnosis of PJP and may consists of clinical features, patient risk factors, disease status, chest radiograph, chest Computed Tomography (CT), Lactate dehydrogenase LDH evaluation, other investigations and lung biopsy [47,48].

LDH Evaluation

Serum LDH levels are generally increased in PJP infection. 90% of HIV patients have shown high range of serum LDH levels. Increased serum LDH levels are seen in immuno-compromised people without HIV infection. Other various factors also may cause to raise the levels in their body. As a result, LDH evaluation is not a confirmed diagnosis of PJP [6].

S-Adenosylmethionine (AdoMet) Technique

P. jirovecii cannot synthesise S-Adenosylmethionine (AdoMet) molecule. AdoMet levels are decreased in patients with PJP infection. Measurement of S-Adenosylmethionine plasma concentrations could give to a new method for PJP diagnosis and also helpful in patient treatment [6,48].

Chest Radiography

Based on the clinical findings and severity of the illness, clinicians refer chest radiography to know the status of the disease in patients. Early mild disease condition, normal chest radiographic findings may be normal. Most of the PJP cases have shown abnormal chest radiographic findings. Chest radiography has shown diffuse bilateral infiltrates predominantly peri-hilar distribution in PJP patients. Other radiographic findings consist of pneumothorax, patchy asymmetric infiltrates, pneumatocele and fine reticular interstitial changes [47].

Computed Tomography (CT): Significant radiology findings of *Pneumocystis* were not seen in chest radiography, but patient's clinical symptoms have shown infection of *Pneumocystis*. When chest X-ray is negative, High Resolution Computed Tomography (HRCT) is very helpful to identify considerable findings. In HIV patients, HRCT is useful to identify findings specifically. CT is the most sensitive radiology method compared to chest X-ray. In patients with *Pneumocystis* infection, CT scan reveals bilateral patchy ground glass appearance. Granular, reticular and cystic lesions are less common features seen in patients with PJP [49].

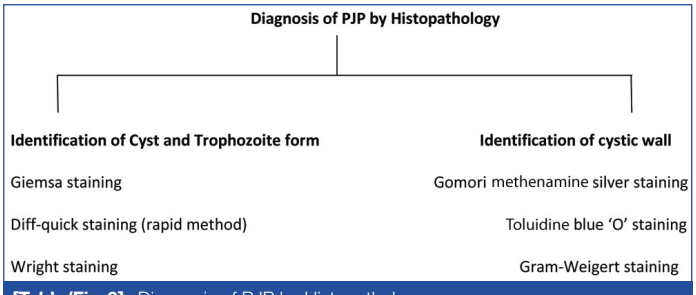
Arterial Blood Gas (ABG) analysis: An ABG analysis is useful marker for evaluating the severity of the PJP infection. ABG level should be measured for the probable adjunctive corticosteroid therapy in patients with hypoxic condition. Usually Alveolar-arterial (A-a) oxygen gradient is raised in PJP infection [4].

Pulmonary Function Tests

Spirometry, Diffusing Capacity for Carbon Monoxide (DLCO)

Clinical Samples: *P. jirovecii* is a lung pathogen. Lung exudates are used for diagnosis of PJP. Clinical samples such as Sputum, Induced sputum, BAL, Pleural fluid and Lung tissue are used for identification of *Pneumocystis* infection.

Staining: Different stains such as Methenamine silver, Calcoflour white, Toluidine blue-O, Acridine orange, Geimsa staining and Diff-Quik staining are used for identification of *Pneumocystis*. Immunofluorescence (IF) staining is rapid, easy to perform, most sensitive and specific method for detection of *Pneumocystis* compared to other conventional methods [38,50]. This staining is very helpful to the patients with low organism load [Table/Fig-3].



[Table/Fig-3]: Diagnosis of PJP by Histopathology.

Serology

Serum markers: Krebs von den Lungen-6 (KL-6) is a mucin-like glycoprotein with high-molecular-weight firmly expressed on Type-2 alveolar pneumocytes and bronchiolar epithelial cells. Levels of KL-6 in serum are a responsive indicator of different categories of interstitial pneumonitis. These levels are high in HIV patients with PJP compared to non-HIV group of patients [51].

Using recombinant antigens of *P. jiroveci* and immuno-enzymatic or immuno-blotting assays have potential application in diagnosis of PJP [52].

In-vitro Cultivation

In-vitro culture system is not yet developed completely. Short time efficient in-vitro methods promote the production of infectious organisms in short period of time. Cell lines like Mink lung cell line Mv 1, human lung fibroblast line HEL and Human lung carcinoma cell line A 549 were usually used for *Pneumocystis* culture and conventional stains, antibodies can be used to identify them [53]. In 2014, first success was achieved by using a three-dimensional air-liquid interface culture system formed by CuFi-8 respiratory epithelial cell line. This is used for cultivation and propagation of *P. jirovecii* directly from the BAL sample [54].

RECENT ADVANCES IN DIAGNOSIS BY MOLECULAR METHODS

Molecular methods have been developed for diagnosis of PJP. PCR assays are most sensitive and specific methods useful for detection of *P. jirovecii* from infected individuals, recurrent PJP patients. PCR assays can be helpful for estimation of prevalence of disease as well as epidemiological purposes. Immuno-compromised individuals without HIV may have low number of pathogen in their lung compared to HIV positive patients. Low number of pathogen burden and patients under chemoprophylaxis may decrease the sensitivity in microscopic methods, in such cases PCR assays are very helpful tools for confirmatory diagnosis [55,56]. Commonly used clinical samples are BAL, sputum, induced sputum, oral washes and pleural fluid. Different types of PCR assays can be used for different gene targets for detection of *Pneumocystis*. Optimised single round touchdown PCR have been used for routine purposes. Nested PCR increases the sensitivity of the test [55].

New Diagnostic methods of *P.jirovecii*: Commercial kits like Myc Assay, AmpliSens, Bio-Evolution PCR, Fast Track Diagnostics (FTD), to detect *P. jirovecii* were studied by different researchers [57-59] by comparing with conventional methods of detection.

Following are few molecular typing methods for detecting *P. jirovecii* [Table/Fig-4] [60]. A flowchart has been devised for diagnosis of the PJP infections [Table/Fig-5] [56,61].

TREATMENT/PREVENTION

Treatment of PJP infection is based on the severity of the illness and diagnosis. Evidence based guidelines should be followed for giving proper treatment. Common antifungal drugs are not effective for PJP infection. PJP should be treated with prescribed medicine. If Treatment is not given, the patient will die with PJP infection. Drug of choice for PJP infection is Trimethoprim-Sulfamethoxazole (TMP-SMX), also known as co-trimoxazole. TMP-SMZ is highly effective drug compared to other drugs because of good penetration, fast clinical response and low cost. TMP-SMZ is a first line medication in both HIV and Non-HIV individuals as a 21 days course.

Treatment in HIV Patients

Mild to Moderate disease: oral medication, 3 divided doses of TMP 15 to 20 mg/kg/day and SMZ 75 to 100 mg/kg/day.

Moderate to severe cases: Intravenous (IV) administration of TMP 15 to 20 mg/kg/day and SMZ 75-100 mg/kg/day for every 6 to 8 hours [3]. Side effects are fever and rash [39]. Intensity of allergies increased by prolonged usage of drug. Alternative drug regimen can be used when TMP-SMZ side effects are severe.

Second Line Drugs for Severe Cases

Clindamycin and Primaquine: In mild and moderate diseased condition, clindamycin and primaquine are good alternative choice for treatment of PJP.

Pentamidine is the most preferred alternative drug regimen to TMP-SMZ [62,63]. Intravenous pentamidine is given at least 14-21 days as a dose of 4 mg/kg/day [64].

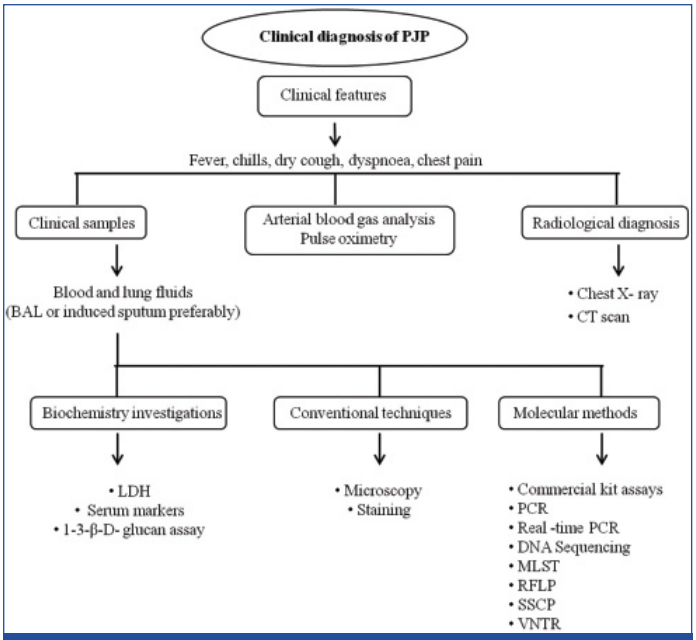
Second Line Drugs for Moderate or Mild Disease

Dapsone is another alternative drug for TMP-SMZ for treating PJP infection. Daily dosage of 100 mg plus trimethoprim (15 mg daily) should be administered [65].

Atovaquone: Another drug regimen for PJP infection. Daily dose of Atovaquone is 1500 mg (twice a day 750 mg), given orally with food. Atovaquone is a good alternative choice for the patients who cannot tolerate TMP-SMZ and dapsone. It has a good tolerating capacity but response of this drug is poor and also expensive [66]. This drug can be used in patients who are intolerant to TMP-SMZ and pentamidine. It is less effective compared to the TMP-SMZ drug regimen.

Sl. No	Method	
1.	Single-locus Sanger DNA sequencing:	This is the most common method for single-locus typing of *P. jirovecii*. This method is accommodating to recognise all well-known or potentially novel sequence variants in the target areas. Still Sanger sequencing is a good typing method compared to other newer methods because of attractive option in many circumstances and also with fast and inexpensive commercial sequencing services.
2.	Multilocus sequence typing (MLST)	MLST involved amplified PCR product followed by DNA sequencing of a number of genes. Almost the entire recognised genetic markers for *P. jirovecii* have been estimated for their attainable to develop an MLST system. Although it is expensive and labour-intensive to amplify and sequence entity loci from individual patients, an effort has been ready to conquer this negative aspect by achieving whichever DNA pooling approaches or real-time amplification of many loci followed by single-base conservatory analysis. These approaches have possible for high-throughput relevance but have not been assessed by different laboratories.
3.	Restriction fragment length polymorphism analysis (RFLP)	This is the most accepted method since 1980s and early 1990s to until now. This method is commonly used for typing of numerous organisms. Advantages of this method consist of in-expensive instruments, less time processing (without hybridization) and a high sensitivity (with hybridization). RFLP method is usually recognised for DHPS gene mutations of *P. jirovecii*. Newly, RFLP was modified to identify polymorphisms of the *P. jirovecii* msg repertoire.
4.	Single-strand confirmation polymorphism analysis (SSCP)	SSCP is a quite easy and convenient method to distinguish nucleotide variations within and between amplicons (~100 to 500 bp). Mainly SSCP is useful for typing of *P. jirovecii*, its capability to discriminate various sequences in patients with co-infections and also possible to done for quick screening of huge numbers of samples per day.
5.	Variable-number tandem-repeat (VNTR) analysis:	Unlike typing methods, this method is helpful to detect nucleotide substitutions or indels in non-repetitive loci. This investigation is to be dependent on enumerated the repeat copy numbers of short tandem repeats, furthermore called as microsatellites. Highest sensitivity is the main advantage of this method for detection of a small population in mixed populations of *P. jirovecii* and which can be observed in up to 92% of patients with PJP.
6.	DNA Sequence Analysis	Commonly molecular typing studies and biodiversity of *Pneumocystis* is done by direct sequence analysis. The mitochondrial large subunit ribosomal RNA locus (mt LSU rRNA), dihydropteroate synthase (DHPS) gene Internal Transcribed Spacer (ITS) regions of the nuclear rRNA operon are helpful for molecular epidemiology applications compared to Superoxide dismutase (SODA) gene loci, thymidylate synthase (TS), EPSP synthase domain of the multi-functional arom gene and the mitochondrial small subunit ribosomal RNA (mt SSU rRNA). These are not highly beneficiary, because of low sequence divergence of *Pneumocystis* [56,61].

[Table/Fig-4]: Molecular typing methods [56,61].



[Table/Fig-5]: Flow chart for diagnosing PJP.

Haematological patients: Daily dose of TMP-SMZ 15-20 mg/kg TMP; 75-100 mg/kg SMZ. Second line drugs -Daily doses of Primaquine 30 mg and clindamycin 600 mg thrice. Daily dose of Pentamidine IV 4 mg/kg. Daily dose of Atovaquone 750 mg twice or thrice. As per ECIL guidelines, currently high dose of TMP-SMZ over two week's period is a treatment of choice in PJP patients with haematological malignancies [67].

Solid organ transplantation patients: Daily dose of TMP-SMZ 15-20 mg/kg; 75-100 mg/kg) should be received by patient with every 6-8 hours time, TMP administered by IV route. Combination with daily dose of prednisolone 40-60 mg should be given twice in hypoxemic patients [68].

RECENT TREATMENT MODALITIES

Treatment Modality with Caspofungin

Li H et al., reported, Caspofungin combined with Clindamycin is alternative treatment for PJP infection when treatment fails with TMP-SMZ and patients intolerant to TMP-SMZ [69].

Caspofungin with TMP-SMZ

Another recent study by Yu B et al., evaluated and reported that combined therapy of Caspofungin with TMP-SMZ is more effective than TMP-SMZ monotherapy in renal transplant patients [70].

Recent study from Japan showed Sulfasalazine is a better treatment choice in Rheumatoid Arthritis (RA) patients due to high preventive effect of drug against PJP [71].

Retroviral Therapies Interfere with Survival Rate of the Patients Infected with PJP

Immune-reconstitution, anti-retroviral therapy is one of the effective way of avoiding PJP in individuals alive with HIV. Anti PJP and anti-retroviral therapies comprise additive or synergistic toxicities. It may lead to delay in the instigation of anti-retroviral therapy until after beginning anti-PCP therapy or in a few cases until after the end of anti-PJP therapy. AIDS succession mortality can be diminished in people with acute opportunistic infections by initiating early antiretroviral therapy. This study showed both therapies were not linked with the side-effects as well as decrease in the effectiveness of antiretroviral therapy. Eventually study concluded that Anti Retro Viral therapies interfere with a reduction in AIDS progression and increase in survival rate [72].

Adjuvant Corticosteroids Affect the Course of Disease in Immuno-compromised Patients

In HIV patients: Early involvement of corticosteroids in AIDS patients with severe PJP infection give results in an impressive survival benefit and reduced the frequency of respiratory failure. Early adjuvant corticosteroid therapy at least 7 days with severe PJP may reduce intensive care unit stay. Moderate to severe PJP, adjunctive corticosteroids initiated at the time of PJP therapy prevented the early decline in oxygenation (arterial-alveolar difference >35 mmHg or an arterial oxygen pressure <70 mmHg) may lead to respiratory failure and death. In children with HIV positive condition, usage of short course corticosteroids may be helpful for management of PJP. In solid organ transplant patients, usage of corticosteroids may be a risk factor and developing PJP. Widely used corticosteroids for management of haematological malignancies may be a risk factor for PJP infection [65,73].

Prophylaxis

Evidence based guidelines play a major role for prophylaxis in certain individuals. Primary prophylaxis should be given for PJP patients with immuno-suppressive conditions including haematological malignancy, AIDS, congenital immunodeficiency, organ transplantation. TMP-SMZ is given as an early treatment prophylaxis in patients with PJP. If side effects are severe with TMP-SMZ, other drugs include Pentamidine, Trimetrexate, Atovaquone, Clindamycin and Primaquine combined with Leucovorin, Dapsone and Caspofungin combined with Clindamycin can be given as alternative drug regimen to the patients with PJP infection.

Indications: Primary prophylaxis is indicated to the HIV patients with CD4 count less than 200 cells/μL [5], oropharyngeal candidiasis and patients with history of other opportunistic infections. Secondary prophylaxis is indicated for patients with a history of PJP.

Clinicians can stop the primary and secondary treatment prophylaxis in patients which had responded to (HAART) with a raise of CD4 count >200 cells/μL. CD4 count monitoring should be crucial for every three months. If CD4 count is <200 cells/μL, prophylaxis should be restarted. This is a safety practice proven by studies. This type of treatment prophylaxis can be helpful to decrease the usage of drug regimen, side effects of drug and variety of drug challenging pathogens [74,75].

DRUG RESISTANCE AND MUTATIONS

Drug resistance is the common problem usually observed in microorganisms. Earlier to the HIV epidemic in developing countries, usage of TMP-SMZ is rare in patients due to toxic effect. Very low drug resistance was reported in those countries [76]. Later TMP-SMZ used as common drug regimen for treatment of PJP. Extensive utilisation of TMP-SMZ and Dapsone in HIV and other immuno-compromised individuals can lead to Sulfa (Sulfonamide or Sulfone) drug resistance. As it is difficult to culture *Pneumocystis*, drug susceptibility testing is not possible.

Wide spread and long term usage of PJP prophylaxis can lead to rising new cases of DHPS mutant strains. The spread of resistant mutants due to genetic mutations and other possible reason may be person-to-person transmission of *P. jirovecii* infection [77,78]. A lot of single base polymorphisms were reported in DHPS of *P. jirovecii*. Substrate binding was affected by these mutations and exhibit resistance to dapsone and sulphonamide. Mutations occurred in cytochrome b gene exhibit atovaquone resistant *P. jirovecii*. Survival rate does not completely depend upon the mutated *P. jirovecii* strains and may be other factors can play a role in HIV patients [79,80]. Evaluation of drug resistance is complicated in HIV patients and finally further advance research is required for strategies development as well as to stop the further increase of resistant strains.

Recent study from Delhi reported frequency of mutations in the DHPS gene of *P. jirovecii* isolates. A novel mutant strain was reported

in 3 cases (25.0%) among 12 cases of infected patients and no mutations in remaining 9 cases. Aforesaid 9 cases, responded very well to the treatment. Therefore, novel DHPS mutant strain is associated with drug resistance and mortality [35].

Very fewer studies conducted on genetic variations in *P. jirovecii* Dihydrofolate reductase (DHFR) gene. Almost 12 of the studies reported, no DHFR mutations were observed. In dissimilarity, about 6 studies were reported, wide-ranging mutations was seen at more than 30 amino acid positions. Through in-vitro testing, recombinant *P. jirovecii* of DHFR enzymes comprised known mutations. Regarding this six DHFR variants were resistant to TMP and may leads to clinical resistance. Few studies conducted on atovaquone resistance. It targets mitochondrial gene cytochrome *b (COB)*. More than 70 isolates revealed different mutations by sequence analysis with only 2 present in more than one isolate. These three drug targets *DHPS*, *DHFR*, and *COB* were also detected in PJP patients with no previous contact to the concerned drugs. These patients may spread mutant *P. jirovecii* strains to other persons. These mutations concurrently with identical nucleotide changes in these genes may be helpful as indicators in epidemiological purposes [60].

Genetic Influences of *P. jirovecii*

Genetic diversity plays a key role in adapting to changing environments, for the survival of a species. Cisse OH et al., investigated the diversity and demographic histories of natural populations of *Pneumocystis,* communicate a disease to humans, rats, and mice [81]. They have collected infected tissues from various geographical settings and carried out whole-genome and large scale multilocus sequencing. They reported rate of evolution, range of population and levels of genetic diversity. This study revealed high rate of infection and natural populations maintain an elevated level of genetic variation in spite of low levels of recombination. Finally, this study results give a dynamic outlook of the evolution of *Pneumocystis* populations and improve indulgent of its transmission [81].

Duggal P et al., examined the effects of a novel non-conservative acidic to basic alter in the third codon of the extracellular domain of CXCR6 on point in time to death after a proven diagnosis of PJP [82]. This study concluded that there is an association among genotype CXCR6 and progress delayed from PJP to death among African-Americans with HIV disease. This study proposed with the aim of CXCR6 may play a role in stage HIV-1 infection and may change the progression to fatality after early infection with PJP [82].

Risk of Evaluation and Outcome in Solid Organ Transplant (SOT) Patients

As per American Society of Transplantation Infectious Diseases Community of Practice guidelines 2019, *P. jirovecii* infection may develop through airborne route or reactivation of previous infection in organ transplant patients. Generally PJP have shown highest risk in first 6 months after organ transplantation. Better prophylaxis drug is TMP-SMZ. No prophylaxis may increase the risk of PJP. Lower death rate in SOT identified as an independent variable associated with a lower death rate and similar to that of AIDS. A deferred diagnosis, resultant in a delay in the beginning of a modified PCP treatment, can contribute in a poor outcome where as the time from admission to beginning of PCP treatment is an independent predictor of death being older, needing oxygen or invasive mechanical ventilation on admission signifies a poor prognosis. A World Health Organisation (WHO) performance standing over 2, an uncontrolled of the underlying infection, a high temperature, hypoalbuminemia, shock, and clinical worsening by day 5 are also associated with raised mortality. Pulmonary co-infections with CMV or herpes simplex virus and an elevated neutrophil count in a BAL sample are also correlated with more severe hypoxemia and higher mortality rates [83,84].

Implications in Chronic Lymphocytic Leukaemia Patients

As per ECIL guidelines, no antifungal treatment is needed for Chronic Lymphocytic Leukaemia. Patients with prolonged neutropaenia (more than 6 months) elderly, advanced and impassive disease necessitate it [67].

CONCLUSION

PJP remains a still major health problem in both HIV and other immuno-compromised individuals. Improper treatment for PJP infection may lead to mortality. HIV cases with HAART may reduce the risk of PJP. Mortality is associated with PJP in both HIV and non-HIV patients. The rate of infection as well as mortality rates was increasing year by year. Major problem in AIDS patients with *Pneumocystis* infection leads to increasing death rate due to failure of drug therapy. Finally, further advance research is required for strategies development as well as to stop further increase of resistant strains. New diagnostic methods and usage of non-invasive respiratory specimens such as oral washes for diagnosis of PJP should be expanded. PCR is the most sensitive method compared to other conventional and serological methods. Using PCR and Sequence analysis can be helpful for estimation of prevalence of disease as well as epidemiological purposes. Following evidence based guidelines will be helpful for giving proper treatment and can reduce the improper usage of drugs and decrease drug resistance. Conducting programmes to public, regarding disease will be helpful for patient management particularly in HIV people suffering with opportunistic infections. There is an urgent need to develop newer drugs and vaccines to eradicate the PJP disease burden.

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REFERENCES

[1] Ricciardi A, Gentilotti E, Coppola L, Maffongelli G, Cerva C, Malagnino V, et al. Infectious disease ward admission positively influences *P. jiroveci* pneumonia (PJP) outcome: A retrospective analysis of 116 HIV-positive and HIV-negative immunocompromised patients. PLoS ONE. 2017;12(5):e0176881.
[2] Mecoli CA, Saylor D, Gelber AC, Christopher-Stine L. *Pneumocystis jirovecii* pneumonia in rheumatic disease: A 20-year single-centre experience. Clin. Exp. Rheumatol. 2017;35(4):671-73.
[3] Truong J, Ashurst JV. *Pneumocystis (Carinii) Jiroveci* Pneumonia. [Updated 2019 Feb 22]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 Jan-. Available from: https://europepmc.org/books/NBK482370;jsessionid=100D61CBCF6B7F6B061C2FD3DB0A60F9
[4] Shelley A Gilroy. *Pneumocystis jiroveci* Pneumonia (PJP) [Updated 2019 Apr 24]. Available from: https://emedicine.medscape.com/article/225976-overview.
[5] Kaplan JE, Hanson D, Dworkin MS, Frederick T, Bertolli J, Lindegren ML, et al. Epidemiology of human immunodeficiency virus-associated opportunistic infections in the United States in the era of highly active antiretroviral therapy. Clin Infect Dis. 2000;30 Suppl 1:S5-14.
[6] George MP, Gingo MR, Morris A. *Pneumocystis (carinii) jirovecii*. Available from *http://www.antimicrobeorg/new/f11asp#top*. Accessed on 04.06.2019.
[7] Amber KT. Balancing the risks and benefits of prophylaxis: a reply to "*Pneumocystis jiroveci* pneumonia in patients treated with systemic immunosuppressive agents for dermatologic conditions". Int J Dermatol. 2017;56(1):e4-e5.
[8] Fillâtre P, Revest M, Belaz S, Robert-Gangneux F, Zahar JR, Roblot F, et al. [Pneumocystosis in non-HIV-infected immunocompromised patients]. Rev Med Interne. 2016; 37(5):327-36.
[9] Antinori A, Maiuro G, Pallavicini F, Valente F, Ventura G, Marasca G, et al. Prognostic factors of early fatal outcome and long-term survival in patients with *Pneumocystis carinii* pneumonia and acquire immunodeficiency syndrome. Eur J Epidemiol. 1993;9:183-89.
[10] Stringer JR, Beard CB, Miller RF, Wakefield AE. A new name (*Pneumocystis jirovecii*) for Pneumocystis from humans. Emerg Infect Dis. 2002;8:891-96.

[11] Ioannidis JP, Cappelleri JC, Skolnik PR, Lau J, Sacks HS. A meta-analysis of the relative efficacy and toxicity of *Pneumocystis Carinii* prophylactic regimens. Arch Intern Med. 1996;156:177-88.

[12] Centers for Disease Control (CDC). *Pneumocystis* pneumonia--Los Angeles. Morb Mortal Wkly Rep. 1981;30:250-52.

[13] Masur H. HIV-Related opportunistic infections are still relevant in 2015. Top Antivir Med. 2015;23(3):116-19.

[14] Rey A, Losada C, Santillán J, Fiorentini F, Schiaffino M, Peroni HJ, et al. *Pneumocystis jiroveci* infection in patients with and without HIV: A comparison. Rev Chilena Infectol. 2015;32:175-80.

[15] Solano L MF, Alvarez Lerma F, Grau S, Segura C, Aguilar A. *Pneumocystis jirovecii* pneumonia: Clinical characteristics and mortality risk factors in an Intensive Care Unit. Med Intensiva. 2015;39:13-19.

[16] Buchacz K, Lau B, Jing Y, Bosch R, Abraham AG, Gill MJ, et al. North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of IeDEA. Incidence of AIDS-Defining Opportunistic Infections in a Multicohort Analysis of HIV-infected Persons in the United States and Canada, 2000-2010. J Infect Dis. 2016;214:862-72.

[17] de Armas Rodriguez Y, Wissmann G, Muller AL. *Pneumocystis jirovecii* pneumonia in developing countries. Parasite. 2011;18:219-28.

[18] Weverling GJ, Mocroft A, Ledergerber B, Kirk O, Gonzales-Lahoz J, d'Arminio Monforte A. Discontinuation of *Pneumocystis carinii* pneumonia prophylaxis after start of highly active antiretroviral therapy in HIV- 1 infection. EuroSIDA Study Group. Lancet. 1999;353:1293-98.

[19] Hughes WT. Current issues in the epidemiology, transmission and reactivation of *Pneumocystis carinii*. Semin Respir Infect. 1998;13:283-88.

[20] Malin AS, Gwanzura LK, Klein S, Robertson VJ, Musvaire P, Mason PR. *Pneumocystis carinii* pneumonia in Zimbabwe. Lancet. 1995;346:1258-61.

[21] Fisk DT, Meshnick S, Kazanjian PH. *Pneumocystis carinii* pneumonia in patients in the developing world who have acquired immunodeficiency syndrome. Clin Infect Dis. 2003;36:70-78.

[22] Singh YN, Singh S, Rattan A, Ray JC, Sriniwas TR, Kumar A. *Pneumocystis carinii* infection in patients of AIDS in India. J Assoc Physicians India. 1993;41:41-42.

[23] Mirdha BR, Guleria R. Comparative yield of different respiratory samples for diagnosis of *Pneumocystis carinii* infections in HIV seropositive and sero-negative individuals in India. South-East Asian J Trop Med Pub Health. 2000;31:473-77.

[24] Merchant RH, Oswal JS, Bhagwat RV, Karkare J. Clinical profile of HIV infection. Indian Pediatr. 2001;38(3):239-46.

[25] Kumarasamy N, Vallabhaneni S, Flanigan TP, Mayer KH, Solomon S. Clinical profile of HIV in India. Indian J Med Res. 2005;121:377-94.

[26] Rajagopalan N, Suchitra JB, Shet A., Khan ZK, Martingarcia J, Nonnemacher MR. Mortality among HIV-Infected patients in resource limited settings: A case controlled analysis of inpatients at a community care center. Am J Infect Dis. 2009;5:219-24.

[27] Shahapur PR, Bidri RC. Recent trends in the spectrum of opportunistic infections in human immunodeficiency virus infected individuals on antiretroviral therapy in South India. J Nat Sc Biol Med. 2014;5:392-96.

[28] Chawla K, Martena S, Gurung B, Mukhopadhyay C, Varghese GK, Bairy I. Role of PCR for diagnosing *Pneumocystis jirovecii* pneumonia in HIV-infected individuals in a tertiary care hospital in India. Indian J Pathol Microbiol. 2011;54:326-29.

[29] Ramesh K, Gandhi S, Rao V. Clinical profile of human immunodeficiency virus patients with opportunistic infections: A descriptive case series study. Int J App Basic Med Res. 2015;5:119-23.

[30] Jairam A, Dassi M, Chandola P, Lall M, Mukherjee D, Hooda AK. *Pneumocystis jiroveci* outbreak in a renal transplant center: Lessons learnt. Indian J Nephrol. 2014;24(5):276-79.

[31] Kaur R, Panda PS, Dewan R. Profile of pneumocystis infection in a tertiary care institute in North India. Indian J Sex Transm Dis AIDS. 2016;37(2):143-46.

[32] Deodhar D, Koshy JM, John M, Oberoi A. Clinical profile of *pneumocystis jirovecii* infection- A comparative study. J Assoc Physicians India. 2018;66(1):28-31.

[33] Jeswani J, Godara S, Bhagat C. Risk factors, clinical manifestations, and outcomes of *Pneumocystis jirovecii* infection in post-renal transplant recipients. J Egypt Soc Nephrol Transplant. 2018;18:112-15.

[34] Shilpa, Andgi A. Clinical profile of opportunistic infections in HIV seropositive patients attending tertiary centre, Raichur, India. Int J Adv Med. 2018;5(6):1369-73.

[35] Singh Y, Mirdha BR, Guleria R, Kabra SK, Mohan A, Chaudhry R, et al. Novel dihydropteroate synthase gene mutation in *Pneumocystis jirovecii* among HIV-infected patients in India: Putative association with drug resistance and mortality. J Glob Antimicrob Resist. 2019;17:236-39.

[36] Kumarasamy N, Venkatesh KK, Devaleenol B, Poongulali S, Yephthomi T, Pradeep A, et al Factors associated with mortality among HIV-infected patients in the era of highly active antiretroviral therapy in southern India. Int J Infect Dis. 2010;14(2):e127-31.

[37] Udwadia ZF, Doshi AV, Bhaduri AS. *Pneumocystis carinii* pneumonia in HIV infected patients from Mumbai. J Assoc Physicians India. 2005;53:437.

[38] Prevost, MC, Escamilla, R, Aliouat, EM, Cere, N, Coudert, et al. *Pneumocystosis* pathophysiology. FEMS Immunol Med Microbiol. 1998;22:123-28.

[39] *Pneumocystis pneumonia*. Fungal Diseases. CDC https://www.cdc.Gov/fungal/diseases/pneumocystis-pneumonia.

[40] Sheikholeslami MF, Sadraei J, Farnia P, Forozandeh Moghadam M, Emadi Kochak H. Co-infection of *Mycobacterium tuberculosis* and *Pneumocystis jirovecii* in the Iranian patients with human immunodeficiency virus. Jundishapur J Microbiol. 2015;8(2):e17254.

[41] Nethathe G, Patel N. Survival after *Pneumocystis jirovecii* pneumonia requiring ventilation: A case report. South Afr J HIV Med. 2016;17(1):474.

[42] Govender S, du Plessis SJ, Ocana GS, Chalkley LJ. Prevalence of *Pneumocystis jirovecii* and *Mycoplasma pneumoniae* in patients presenting with pneumonia at hospitals in Port Elizabeth, Southern African Journal of Epidemiology and Infection. 2008;23:2:21-24.

[43] Sundsted KK, Syed H, Burton MC. 69-year-old woman with dyspnea and cough productive of white sputum. Mayo Clin Proc. 2011;86(12):1225-28.

[44] Simkins J, Corrales-Medina V, Symes S, Dickinson G. Pulmonary embolism in patients with acquired immunodeficiency syndrome presenting with clinical picture of *Pneumocystis jiroveci pneumonia*: report of two cases. Scand J Infect Dis. 2007;39(6-7):634-36.

[45] Čurić K, Poljak M, Ihan A, Tomažič J. Very recent HIV infection accompanied by P*neumocystis jirovecii* pneumonia and *Mycobacterium avium* complex immune reconstitution inflammatory syndrome: A case report. *Acta* Dermatovenerol Alp Pannonica Adriat. 2016;25(3):57-58.

[46] Tasaka S. Pneumocystis pneumonia in human immunodeficiency virus-infected adults and adolescents: Current concepts and future directions. Clin Med Insights Circ Respir Pulm Med. 2015;9(Suppl 1):19-28.

[47] Carmona EM, Limper AH. Update on the diagnosis and treatment of *Pneumocystis pneumonia*. Therapeutic Advances in Respiratory Disease, 2011;41-59.

[48] Skelly M, Hoffman J, Fabbri M, Holzman RS, Clarkson AB, Jr., Merali S. S-adenosylmethionine concentrations in diagnosis of *Pneumocystis carinii* pneumonia. Lancet. 2003;361:1267-68.

[49] Hidalgo A, Falcó V, Mauleón S, Andreu J, Crespo M, Ribera E. Accuracy of high-resolution CT in distinguishing between *Pneumocystis carinii* pneumonia and non-*Pneumocystis carinii* pneumonia in AIDS patients. Eur Radiol. 2003;13(5):1179-84. Epub 2002 Sep 25.

[50] Touhali IS, Ibrahim AAF, Dawood HN. Conventional methods for the diagnosis of *pneumocystis jiroveci* in immunocompromised Iraqi patients. Iraqi JMS;14:80-87.

[51] Tasaka S, Tokuda H. Recent advances in the diagnosis of *Pneumocystis jirovecii* pneumonia in HIV-infected adults. Expert Opin Med Diagn. 2013;7(1):85-97.

[52] Tomás AL, Cardoso F, Esteves F, Matos O. Serological diagnosis of pneumocystosis: production of a synthetic recombinant antigen for immunodetection of *Pneumocystis jirovecii* . Sci Rep. 2016;6:36287.

[53] Contini C, Mastrantoni S, Romani R, Cultera R, Delia S. Evidence of *Pneumocystis carinii* in cell line cultures infected with peripheral blood mononuclear cells isolated from AIDS patients with *P. carinii* pneumonia. J Med Microbiol. 1995;42:394-98.

[54] Lewis White P, Backx M, Barnes RA. Diagnosis and management of *Pneumocystis jirovecii* infection, Expert Review of Anti-infective Therapy. 2017;15(5):435-47.

[55] Helweg-Larsen J, Jensen JS, Dohn B, Benfield TL, Lundgren B. Detection of *Pneumocystis* DNA in samples from patients suspected of bacterial pneumonia-A case-control study. BMC Infect Dis. 2002;2:28. Epub 2002 Nov 25.

[56] Durand-Joly I, Chabé M, Soula F, Delhaes L, Camus D, Dei-Cas E. Molecular diagnosis of *Pneumocystis* pneumonia. FEMS Immunol Med Microbiol. 2005;45(3):405-10.

[57] McTaggart LR, Wengenack NL, Richardson SE. Validation of the MycAssay Pneumocystis kit for detection of *Pneumocystis jirovecii* in bronchoalveolar lavage specimens by comparison to a laboratory standard of direct immunofluorescence microscopy, real-time PCR, or conventional PCR. J Clin Microbiol. 2012;50(6):1856-59.

[58] Sasso M, ChastangDumas E, Bastide S, Alonso S, Lechiche C, Bourgeois N, Lachaud L. Performances of four real-time PCR assays for diagnosis of *Pneumocystis jirovecii* pneumonia. J Clin Microbiol. 2016;54:625-30.

[59] Hoarau G, Le Gal S, Zunic P, Poubeau P, Antok E, Jaubert J, Nevez G, Picot S. Evaluation of quantitative FTD- *Pneumocystis jirovecii* kit for Pneumocystis infection diagnosis. Diagn Microbiol Infect Dis. 2017;89(3):212-17.

[60] Ma L, Cissé OH, Kovacs JA. A molecular window into the biology and epidemiology of pneumocystis spp. Clin Microbiol Rev. 2018;31(3).

[61] Beard CB, Roux P, Nevez G, Hauser PM, Kovacs JA, Unnasch TR, et al. Strain typing methods and molecular epidemiology of *Pneumocystis pneumonia*. Emerg Infect Dis. 2004;10(10):1729-35.

[62] Sattler FR, Cowan R, Nielsen DM, Ruskin J. Trimethoprim-sulfamethoxazole compared with pentamidine for treatment of *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. A prospective, non crossover study. Ann Intern Med. 1988;109:280-87.

[63] Wharton JM, Coleman DL, Wofsy CB, Luce JM, Blumenfeld W, Hadley WK, et al. Trimethoprim-sulfamethoxazole or pentamidine for *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. A prospective randomized trial. Ann Intern Med 1986;105:37-44.

[64] Limper AH, Know KS, Sarosi GA, Ampel NM, Bennett JE, Catanzaro A et al. American Thoracic Society Working Group. An official American Thoracic Society statement: Treatment of fungal infections in adult pulmonary and critical care patients. AJRCCM. 2011;183:96-128.

[65] White PL, Price JS, Backx M. Therapy and Management of *Pneumocystis jirovecii* Infection. J Fungi (Basel). 2018;4(4):127.

[66] Hughes W, Leoung G, Kramer F, Bozzette SA, Safrin S, Frame P, et al. Comparison of atovaquone (566C80) with trimethoprim-sulfamethoxazole to treat *Pneumocystis carinii* pneumonia in patients with AIDS. N Engl J Med. 1993;328:1521-27.

[67] Maertens J, Cesaro S, Maschmeyer G, Einsele H, Donnelly JP, Alanio A, et al. 5th European Conference on Infections in Leukaemia (ECIL-5), a joint venture of the European Group for Blood and Marrow Transplantation (EBMT), the European Organisation for Research and Treatment of Cancer (EORTC), the Immunocompromised Host Society (ICHS) and the European LeukemiaNet (ELN). ECIL guidelines for preventing *Pneumocystis jirovecii* pneumonia in patients with haematological malignancies and stem cell transplant recipients. J Antimicrob. Chemother. 2016;71:2397-2404.

[68] Kosaka M, Ushiki A, Ikuyama Y, Hirai K, Matsuo A, Hachiya T, et al. A four-center retrospective study of the efficacy and toxicity of low-dose trimethoprim sulfamethoxazole for the treatment of pneumocystis pneumonia in patients without HIV infection. Antimicrob Agents Chemother. 2017;61(12).
[69] Li H, Huang H, He H. Successful treatment of severe Pneumocystis pneumonia in an immunosuppressed patient using caspofungin combined with clindamycin: A case report and literature review. BMC Pulm Med. 2016;16(1):144.
[70] Yu B, Yang Y, Ye L, Xie X, Guo J. Comparison of caspofungin and trimethoprim-sulfamethoxazole combination therapy with standard monotherapy in patients with *Pneumocystis jiroveci* pneumonia following kidney transplantation: A retrospective analysis of 22 cases. Int J Clin Exp Med. 2017;10(1):1234-42.
[71] Nunokawa T, Yokogawa N, Shimada K, Sugii S, Nishino J, Gosho M, et al Prophylactic effect of sulfasalazine against Pneumocystis pneumonia in patients with rheumatoid arthritis: A nested case-control study. Semin Arthritis Rheum. 2019;48(4):573-78.
[72] Zolopa A, Andersen J, Powderly W, Sanchez A, Sanne I, Suckow, et al. Early antiretroviral therapy reduces AIDS progression/death in individuals with acute opportunistic infections: A multicenter randomized strategy trial. PLoS ONE. 2009;4, e5575.
[73] Cooley L, Dendle C, Wolf J, Teh BW, Chen SC, Boutlis C et al . Consensus guidelines for diagnosis, prophylaxis and management of *Pneumocystis jirovecii* pneumonia in patients with haematological and solid malignancies. 2014. Intern Med J. 2014;44(12b):1350-63.
[74] Furrer H, Egger M, Opravil M. Discontinuation of primary prophylaxis against *Pneumocystis carinii* pneumonia in HIV-1-infected adults treated with combination antiretroviral therapy. Swiss HIV Cohort Study. N Engl J Med. 1999;340:1301-06.
[75] Ledergerber B, Mocroft A, Reiss P, Furrer H, Kirk O, Bickel M, et al. Eight European Study Groups. Discontinuation of secondary prophylaxis against *Pneumocystis carinii* pneumonia in patients with HIV infection who have a response to antiretroviral therapy. Eight European Study Groups. N Engl J Med. 2001;344(3):168-74.
[76] Harris JR, Marston BJ, Sangrujee N, DuPlessis D, Park B. Cost-effectiveness analysis of diagnostic options for pneumocystis pneumonia (PCP). PLoS One. 2011;6(8):e23158.
[77] Helweg-Larsen J, Benfield TL, Eugen-Olsen J, Lundgren JD, Lundgren B. Effects of mutations in Pneumocystis carinii dihydropteroate synthase gene on outcome of AIDS-associated *P. carinii* pneumonia. Lancet. 1999;354(9187):1347-51.
[78] Kazanjian P, Armstrong W, Hossler PA, Burman W, Richardson J, Lee CH, et al. *Pneumocystis carinii* mutations are associated with duration of sulfa or sulfone prophylaxis exposure in AIDS patients. J Infect Dis. 2000;182(2):551-57.
[79] Friaza V, Morilla R, Respaldiza N, de la Horra C, Calderón EJ. *Pneumocystis jiroveci* dihydropteroate synthase gene mutations among colonized individuals and Pneumocystis pneumonia patients from Spain. Post grad Med. 2010;122(6):24-28.
[80] Baggish AL, Hill DR; Antiparasitic agent atovaquone. Antimicrob Agents Chemother. 2002;46 (5):116373.
[81] Cisse OH, Ma L, Wei Huang D, Khil PP, Dekker JP, Kutty G, et al. Comparative population genomics analysis of the mammalian fungal pathogen pneumocystis. MBio. 2018;9:e00381-18.
[82] Duggal P, An P, Beaty TH, Strathdee SA, Farzadegan H, Markham RB, et al. Genetic influence of CXCR6 chemokine receptor alleles on PCP-mediated AIDS progression among African Americans. Genes Immun. 2003;4(4):245-50.
[83] Iriart X, Bouar ML, Kamar N, Berry A. Pneumocystis pneumonia in solid-organ transplant recipients. J Fungi (Basel). 2015;1(3):293-331. Review.
[84] Fishman JA, Gans H. AST Infectious Diseases Community of Practice. *Pneumocystis jiroveci* in solid organ transplantation: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019:e13587.

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