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Pneumocystis jirovecii in Respiratory Tract of Immunocompromised Patients: A Study in a Tertiary Care Center in Bangalore, South India

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ABSTRACT

Introduction: *Pneumocystis jirovecii* pneumonia (PCP) remains a significant cause of pneumonia among immunocompromised individuals, despite a decline in prevalence with the advent of antiretroviral therapy (ART) for Human Immunodeficiency Virus (HIV). This study aimed to evaluate and compare the diagnostic accuracy of four distinct staining techniques for PCP in respiratory specimens. We assessed the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of these techniques against the gold standard Gomori Methenamine Silver stain (GMS), in order to identify the most effective method for diagnosing PCP. **Methods:** In a prospective observational study, we collected induced sputum (IS) and BAL samples from 100 immunocompromised patients and examined them microscopically for *P. jirovecii* cysts. We employed four staining methods for detection: Calcofluor White, Modified Toluidine Blue, Wright's stain, and Gomori Methenamine Silver stain. **Results:** The combination of Modified Toluidine Blue, Calcofluor White, and Wright's stains detected *P. jirovecii* cysts in 5% of the study population. The sensitivity of the staining methods was: 80% for Modified Toluidine Blue, 40% for Calcofluor White, and 20% for Wright's, compared to the Gomori Methenamine Silver (GMS) stain, which was used as the gold standard. All the staining methods exhibited equivalent specificity (100%). **Conclusion:** The Modified Toluidine Blue stain is a viable alternative to the Gomori Methenamine Silver stain due to its simplicity, speed, and applicability in resource-limited settings. The low prevalence of *P. jirovecii* in this study population suggests that routine cotrimoxazole prophylaxis may be effective in reducing the incidence of *P. jirovecii* pneumonia among HIV patients.

INTRODUCTION

Pneumocystis jirovecii (P. jirovecii) pneumonia, also known as Pneumocystis pneumonia (PCP) or pneumocystosis, is a rapidly progressive and severe fungal infection of the lungs characterized by the presence of *P. jirovecii* cysts [1, 2]. It primarily affects individuals with compromised immune systems, including patients undergoing immunosuppressive therapy for organ transplantation and malignancies [3], as well as premature infants and patients with acquired immunodeficiency syndrome (AIDS) [4, 5]. As *P. jirovecii* is non-cultivable, definitive diagnosis requires morphological confirmation of the organism through techniques like histopathology [6, 7]. India is experiencing an increasing burden of both HIV and PCP infections [8]. In resource-limited settings, diagnosing pneumocystosis is challenging due to limited access to diagnostic facilities and the presence of more prevalent and virulent infections like tuberculosis, which can mask the symptoms of pneumocystosis [9, 10].

In this study, we evaluated the diagnostic performance of four staining methods - Calcofluor White, Modified Toluidine Blue, Wright's, and Gomori Methenamine Silver (GMS) - in detecting *P. jirovecii* cysts in respiratory tract specimens, with GMS serving as the reference standard. We assessed the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each method by comparing them to the GMS.

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MATERIAL AND METHODS

Sample collection. We prospectively collected 100 IS and BAL samples from immunocompromised patients admitted to the Medicine Department of St. John's Medical College Hospital, Bangalore, who were suspected of having *P. jirovecii* pneumonia based on clinical and radiological findings. We then examined these 100 samples for the presence of *P. jirovecii* cysts using the four staining methods described in the 'Staining Methods' section.

Induced sputum collection. Trained healthcare professionals in the Department of Medicine collected IS samples from patients after a standardized mouthwash procedure with 1% sodium bicarbonate solution to reduce oral flora. Patients were then instructed to perform standardized oral hygiene, followed by inhalation of a nebulized solution of 3% saline in water (3 mL) using an ultrasonic nebulizer. Finally, they expectorated sputum into a sterile container.

BAL collection. A pulmonologist performed bronchoscopies using a flexible fiber-optic bronchoscope (Olympus, Germany) to collect BAL samples. The samples were immediately transferred to sterile containers and centrifuged to obtain a cell pellet for smear preparation. If a delay in processing exceeded 1 h, both IS and BAL samples were stored at 4°C until processing could be completed.

Calcofluor White stain (CW). A sediment drop from the IS or BAL samples was placed on a glass slide. When *P. jirovecii* cysts were scarce, one drop of CW solution was added, followed by one drop of 10% potassium hydroxide (KOH). The slide was then covered with a coverslip and allowed to react at 20-25°C for 5 min. Subsequently, the slide was examined under a fluorescence microscope equipped with a 365 nm excitation filter, which enhances the visibility of CW-stained structures. *P. jirovecii* cysts appeared as bright, apple-green/white structures against a dark background, allowing for their identification [11].

Modified Toluidine Blue 'O' stain. Slides were submerged in a solution of 1% sulfuric acid and 1% acetic acid for 10 min, repeated 2-3 times. After gentle rinsing with tap water for 2 min, excess water was blotted, and the slides were air-dried at 20-25°C for 3-5 min. The slides were then flooded with 1% Toluidine Blue reagent, incubated for 3 min, and rinsed with absolute alcohol. *P. jirovecii* cysts were identified under a light microscope using 10x and 40x magnification. Upon oil immersion and high magnification, the cysts appeared as distinct purple structures against a bluish background, often clustered together [12].

Wright's stain (Diff Quik, Modified Giemsa). Air-dried smears were flooded with Wright's stain and incubated for 1 min, followed by fixation with methanol. An equal volume of distilled water was added until a faint pink color appeared, indicating optimal staining. The diluted stain was incubated for 2.5-5 min, ensuring even distribution by gently tilting the slide. The slide was then rinsed with phosphate-buffered saline (PBS, pH 6.4) until the stain was completely removed. *P. jirovecii* cysts and trophozoites were identified using Wright's stain method, characterized by light purple cytoplasm and darkly stained, central, round nuclei at high magnification. Clumps of cysts or trophozoites embedded within a proteinaceous matrix were often observed [13, 14].

Gomori methenamine silver stain (GMS). Air-dried smears, previously fixed in methanol, were treated with 5% chromic acid for 1 h. The slides were then rinsed with gentle flowing water for 5 min, followed by three rinses with fresh distilled water. Next, they were incubated in a Silver Methenamine solution at 37-45°C for 1-3 h or at 58-60°C for 30 min, until a faint golden-brown color appeared. The slides were then rinsed thoroughly with distilled water (5-6 rinses, 3-5 min each). After rinsing, slides were counterstained with light green solution for 0.5-1 min to enhance contrast and visualization of nuclei and other structures (optional). The slides were then dehydrated through two immersions in 95% absolute alcohol, followed by two changes of xylene for complete tissue clearing. Finally, the slides were mounted with coverslips and examined under a light microscope using a 100x oil immersion objective for *P. jirovecii* cysts and trophozoites. The GMS stain highlighted characteristic features: cyst walls, clusters surrounded by foamy material, and golden-brown cytoplasm against a pale green background [15].

Quality control. To ensure accuracy and consistency, positive control smears prepared from *Candida* cultures were included in every batch of smears, regardless of the staining method used. This quality control measure verified the proper functioning of reagents, staining techniques, and microscopy. The performance of three staining methods (Calcofluor white, modified toluidine blue, and Wright's stain) was evaluated and compared to the reference standard, GMS stain, which is widely recognized as the gold standard for *P. jirovecii* detection [4].

Statistical methods. Fisher's Exact test was employed to determine the statistical significance of differences in categorical variables between two or more groups. The sensitivity and specificity of each staining method were calculated and compared to the GMS standard to evaluate their diagnostic performance. Data analysis was performed using SPSS version 15.0, a statistical software program.

Ethical considerations. This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Ethics Committee (IEC) of St. John's Medical College (SJMC) under reference number TH-206/2023. The study protocol was initially reviewed and approved by the Dissertation Screening Committee of SJMC on 17th September 2008, and subsequently ratified

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by the IEC on 19th July 2023. Informed consent was obtained from all participants (or their legally authorized representatives) prior to enrollment in the study. All data were de-identified and securely stored to ensure participant confidentiality.

RESULTS

Study population. A total of 100 immunocompromised patients, who fulfilled the predetermined inclusion criteria, were recruited from St. John's Medical College Hospital over a one-year period (October 2011 to December 2012).

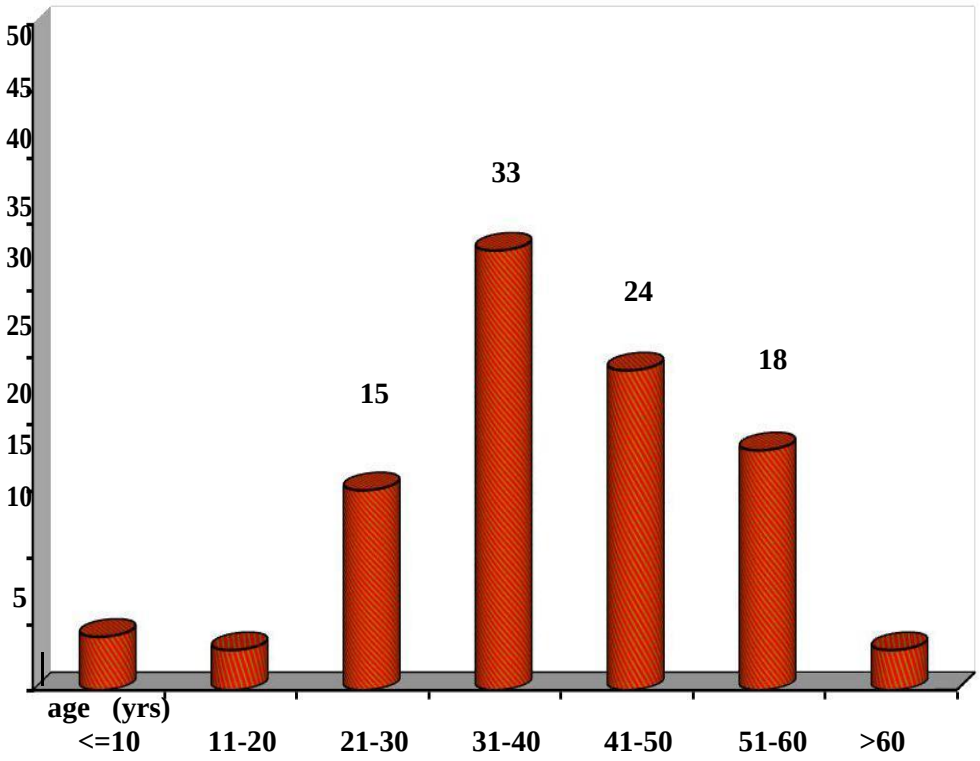

Fig. 1. Age distribution of the study population

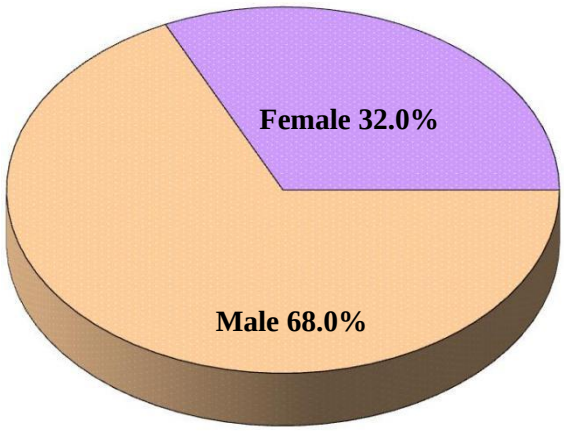

Fig. 2. Distribution of the participants by gender

Demographics. The majority of patients were male (68 males, 32 females), with a male-to-female ratio of 2.1:1. Age: The patient age ranged from 18 to 65 years, with the highest frequency observed in the 31-40 year age group (Fig 1 and Fig 2).

Most specimens (77%) were obtained from induced sputum, while 20% were obtained from BAL samples. Additionally, three patients provided both induced sputum and BAL samples.

[DOI: 10.61186/JoMMID.12.1.67]

Table 1. Frequency distribution of samples by type

Sample type	Number	%
Induced sputum	77	77.0
Bronchoalveolar lavage	20	20.0
Both	3	3.0
Total	100	100.0

Table 2. Included patient characteristics in the study

Investigations	Criteria	Number of samples (n=100)	Percentage
HIV status	Negative	**14**	14.0
	Positive	**86**	86.0
	Absent	42	42.0
Opportunistic infections	Present	58	58.0

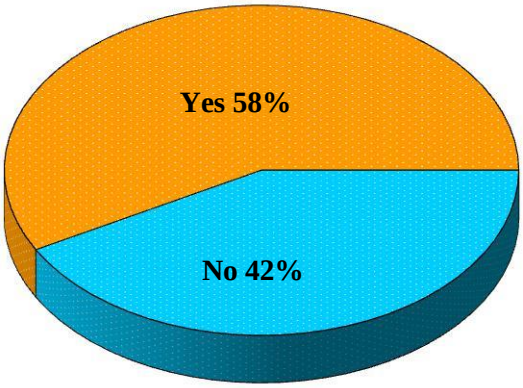

Fig. 3. Pie chart representation of the percentage of opportunistic infections

Immunosuppression. The majority of patients (86%, n=86) were immunosuppressed due to HIV infection. The remaining 14% (n=14) had other underlying conditions leading to immunosuppression, including: renal transplantation (5 patients), chemotherapy (3 patients), autoimmune hemolytic anemia (4 patients), multiorgan dysfunction (4 patients), and idiopathic thrombocytopenic purpura (2 patients).

Table 3. Treatment received by the study population: types of administrated treatments

Treatment	Criteria	Number of samples	%
On anti-retroviral therapy (n=86)	No	38	38%
	Yes	48	48%
On prophylaxis for PCP (n=100)	No	20	20%
	Yes	80	80%

Among the 86 HIV-positive patients, 48 (55.8%) were receiving antiretroviral therapy (ART), while the remaining 38 (44.2%) were not receiving ART or *P. jirovecii* prophylaxis. Of the 48 patients receiving ART, 32 (66.7%) received trimethoprim-sulfamethoxazole (TMP-SMX) as prophylaxis.

Table 4. Conducted laboratory investigations

Investigations	Criteria	No of samples	%
Lymphocyte count (n=98)	Normal (20-45%)	23	23.5
	Lymphopenia (<20%)	75	76.5
CD4 (n=74)	Normal (>250cells/µL)	10	13.5
	Low (<250cells/ µL)	64	86.5
LDH (n=56)	Normal (100-190U/L)	3	5.5
	Raised (>190 U/L)	53	94.6

A low lymphocyte count was observed in 76% (75/98) of patients. Of the 74 patients who had CD4 counts measured, 86.5% (64/74) had a count less than 250 cells/µL. Furthermore, 94.6% (53/56) of the patients who had lactate dehydrogenase (LDH) levels measured had elevated levels.

Chest X-ray abnormalities were observed in 70% (n = 70) of patients, characterized by bilateral interstitial infiltrates, consolidation, reticulonodular patterns, and patchy opacities. A total of 30% (n = 30) of patients had normal chest X-ray results. Additionally, 42% (n = 42) of patients exhibited hypoxia, defined as oxygen saturation below 95%.

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Table 5. Conducted additional laboratory investigations

Investigations	Criteria	No. of patients	%
X-ray (n=80)	Normal	24	30.0
	Abnormal	56	70.0
CT-USG(n=100)	Normal	87	87.0
	Abnormal	13	13.0
ABG analysis (n=73)	Hypoxia absent	42	57.5
(po<70mmHg)	Hypoxia present	31	42.5

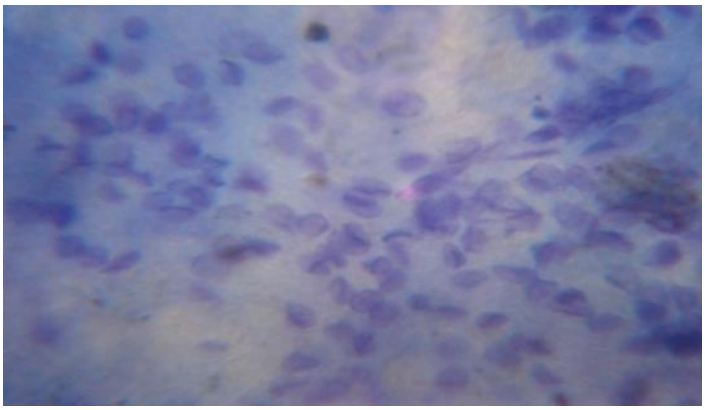

Fig. 4. Visualization of *P. jirovecii* cysts in Modified Toluidine 'O' Blue Stain (100×)

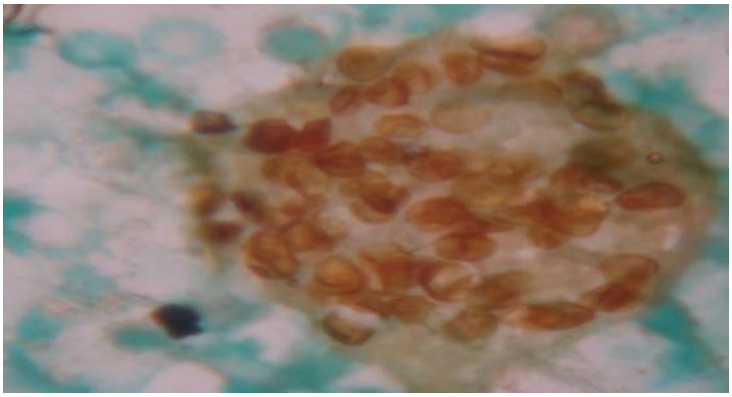

Fig. 5. *P. jirovecii* cysts in Gomori Methenamine silver stain (100× Magnification)

Table 6. Detection of *P. jirovecii* in respiratory tract specimens using four staining methods

Staining methods	*P. jirovecii*	Number of samples (n = 100)	%
Calcofluor White Stain	Absent	98	98.0
	Present	2	2.0
Modified Toluidine O Blue Stain	Absent	96	96.0
	Present	4	4.0
Wright's stain	Absent	99	99.0
	Present	1	1
Grocott's methenamine silver stain (GMS)	Absent	95	95.0
	Present	5	5.0

The diagnostic performance of the stains was as follows: Calcofluor white stain exhibited 40% sensitivity, 100% specificity, 96.94% NPV, and 100% PPV. Toluidine O blue stain demonstrated 80% sensitivity, 100% specificity, 98.95% NPV, and 100% PPV. Finally, Wright's stain displayed 20% sensitivity, 100% specificity, 95.95% NPV, and 100% PPV.

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DISCUSSION

This study aimed to evaluate the diagnostic performance of four staining methods for detecting *P. jirovecii* cysts in respiratory tract specimens, using GMS as the reference standard. Our study observed a lower mean age (33 years) and a more balanced male-to-female ratio (2.1:1) compared to a previous study in Mumbai, which reported a mean age of 40.1 years (range: 18-62 years) and a male-to-female ratio of 5.4:1 [16]. These differences suggest that our study population may have distinct demographic characteristics that could impact the prevalence and presentation of *P. jirovecii* infections.

The mean age of our study population was 33 years, consistent with findings from previous studies conducted in Mumbai (mean age: 40.1 years) and Delhi (mean age: 35 years) [6]. This consistency in age demographics across studies suggests that *P. jirovecii* infections may disproportionately affect individuals within a specific age range, warranting further exploration of this trend and its potential implications for patient care. This study, focusing on a total of 100 immunocompromised patients, utilized microscopic examination to detect *P. jirovecii* cysts in 3 out of 80 patient samples and 2 out of 20 BAL samples. A similar study by Usha *et al*. (2005) in Chennai reported nine positive results for *P. jirovecii* in IS samples [17]. These findings highlight the importance of continued research into the prevalence and characteristics of *P. jirovecii* infections in immunocompromised populations, particularly in different geographic regions.

A previous study investigating the diagnostic value of induced sputum for PCP in AIDS patients evaluated a total of 32 cases. Among them, induced sputum examination using microscopy detected 14 cases, demonstrating a sensitivity of 56% for diagnosing PCP [18]. This finding highlights the potential utility of induced sputum examination as a diagnostic tool for PCP, particularly in resource-limited settings where more invasive procedures may not be feasible. In line with previous studies conducted in India [19], our investigation primarily utilized IS or BAL samples for *P. jirovecii* detection. Among the 86 HIV-positive patients in this study, 48 were receiving highly active antiretroviral therapy (HAART), and 80 received cotrimoxazole prophylaxis, specifically trimethoprim-sulfamethoxazole (TMP-SMX). The widespread use of TMP-SMX prophylaxis in HIV-positive individuals is likely a contributing factor to the observed detection rate of *P. jirovecii* in our study, highlighting the importance of continued monitoring and adaptation of prophylactic strategies in this population.

A study by Dworkin *et al*. (2001) demonstrated that TMP-SMZ prophylaxis offered substantial protection against PCP [20]. It is well-established that the affordability and efficacy of TMP-SMX have significantly contributed to the management of HIV infection in India, leading to significant reduction in HIV-associated morbidity and mortality [21]. Our study identified a 5% prevalence of *P. jirovecii* pneumonia (PJP), with a higher prevalence observed among HIV-positive patients compared to the renal transplant recipient. This finding is comparable to the 6.1% incidence of PCP reported by Kumaraswamy *et al*. (2003) in a study population with a similar mean CD4 cell count of 87 cells/µL [22]. The similarity in prevalence rates between our study and Kumaraswamy et al.'s study suggests that the risk factors for PJP may be consistent across different immunocompromised populations, highlighting the need for continued vigilance and effective prophylactic measures in these groups.

Several studies, including one by Branten *et al*. (1995), have highlighted the increased risk of PCP in post-transplant patients. They documented a specific incidence of 1.1% during 1990-1991 to 11.5% in 1991-1992 [23, 24]. Comparable results were reported in a study conducted by Udwadia *et al*. (2005) in Mumbai [16]. These findings suggest that PCP is a significant complication in post-transplant patients. To assess the diagnostic accuracy of various methods in our study, we calculated sensitivity, specificity, PPV, and NPV for the five positive cases using three distinct staining methods, with the GMS stain designated as the gold standard, and compared them to establish the most reliable diagnostic approach for our patient population. The Calcofluor white stain exhibited a sensitivity of 40%, specificity of 100%, NPV of 96.94%, and PPV of 100%. The Modified Toluidine 'O' Blue stain demonstrated a sensitivity of 80%, specificity of 100%, NPV of 98.95%, and PPV of 100%. Finally, Wright's stain revealed a sensitivity of 20%, specificity of 100%, NPV of 95.95%, and PPV of 100%. This comparison revealed that the Modified Toluidine 'O' Blue stain was the most sensitive and reliable method for diagnosing *P. jirovecii* in our patient population.

In our study, *P. jirovecii* was detected in five out of 100 immunocompromised patients, using GMS staining, with three cases identified in IS and two BAL samples. These five cases served as the reference for evaluating the performance of other staining methods. Toluidine O Blue staining, Calcofluor White staining, and Wright's staining identified four, two, and one of these five cases, respectively. GMS staining demonstrated the highest detection rate (80%), followed by Toluidine O Blue (40%) and Calcofluor White (20%). GMS staining served as the gold standard in our study due to its 100% sensitivity and established accuracy. The efficacy of alternative staining methods was evaluated by comparing their performance to that of the GMS stain. Procop *et al*. (2004) reported comparable diagnostic accuracy for GMS staining, achieving a sensitivity of 79.4%, specificity of 99.2%, positive predictive value (PPV) of 90%, and negative predictive value (NPV) of 90% [11]. These results are comparable to our findings, supporting the use of GMS staining as a reference for evaluating the performance of other staining methods in the detection of *P. jirovecii*.

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Supporting the high sensitivity of GMS staining, Cregan *et al*. (1990) reported detection rates of 92% in sputum and 86% in BAL samples [4]. Another study compared different methods for identifying *P. jirovecii* in pulmonary aspirates and found that 11 out of 29 samples tested positive using GMS stain, which was more sensitive than the other methods tested [14].

Compared to the gold standard GMS staining, Calcofluor White staining exhibited a lower sensitivity (40%) in our study, identifying *P. jirovecii* in two out of five cases. This highlights a potential limitation of Calcofluor White, as its sensitivity might be compromised by the presence of other yeasts in respiratory samples, potentially leading to misdiagnosis or underdiagnosis of *P. jirovecii*. This underscores the importance of using a combination of staining methods or more specific diagnostic tests to ensure accurate diagnosis and appropriate treatment. Vickie *et al*. (1995) compared the diagnostic accuracy of Calcofluor white stain to Toluidine blue O (TBO) and Giemsa stains in a study of 45 BAL specimens. Calcofluor white staining demonstrated high concordance with TBO and Giemsa, with positive results in 95% (21/22) of TBO/Giemsa-positive samples and negative results in all 23 TBO/Giemsa-negative samples [25, 26]. In a study by Procop *et al*. (2004), the sensitivity, specificity, PPV, and NPV of CW staining were 73.8%, 99.6%, 90%, and 90%, respectively [11]. CW staining is a diagnostic technique that utilizes Calcofluor as its active ingredient. This fluorescent stain binds specifically to beta-linked polysaccharides like chitin and cellulose, which are components of fungal cell walls, allowing for the detection of fungi in clinical specimens like respiratory samples [11]. When viewed under a fluorescence microscope with a UV filter (*e.g.*, 365 nm excitation and 432 nm emission), the Calcofluor white-stained fungal elements exhibit characteristic bright green fluorescence due to the binding of the stain to the fungal cell wall components. This simplicity, ease of use, and high specificity make CW staining a valuable tool for routine fungal detection in respiratory samples within diagnostic laboratories.

Our study found the modified Toluidine Blue O (TBO) stain to have a sensitivity of 80%, identifying *P. jirovecii* in 4 out of 5 positive cases. This aligns with findings from other studies, suggesting its potential as a reliable diagnostic tool for detecting *P. jirovecii* in immunocompromised patients [14].

Ng *et al*. (1990) reported a detection rate of 71% (75 out of 182 specimens) using a modified Toluidine Blue O (TBO) stain for *P. jirovecii* in respiratory samples [27]. Usha *et al*. (2000) compared the diagnostic efficacy of three staining methods for detecting *P. jirovecii* in respiratory samples from 32 AIDS patients with respiratory illnesses. Among the employed methods, Giemsa staining identified the parasite in 4 (12.5%) samples, Toluidine Blue staining in 7 (21.87%) samples, and indirect immunofluorescence in 9 (28.12%) samples, demonstrating the highest detection rate [18]. These findings suggest that indirect immunofluorescence may be a more sensitive diagnostic tool for detecting *P. jirovecii* in immunocompromised patients, particularly those with respiratory illnesses. However, it is important to note that the study had a small sample size and further research is needed to confirm these results.

A study at Christian Medical College and Hospital, Vellore, examined 204 respiratory samples for *P. jirovecii* using microscopic examination of Toluidine O and Giemsa stains. The analysis detected *P. jirovecii* cysts in 24 samples (11.8%) [9]. However, it is important to note that the sensitivity and specificity of these stains may vary depending on the population studied and the expertise of the microscopist. Toluidine Blue O stain can be routinely employed in laboratories to detect specific cellular components or microorganisms. The staining procedure involves treating the sample with a sulfation reagent, which effectively removes background debris such as mucus, allowing for enhanced contrast and easier visualization of *P. jirovecii* cysts. The cysts appear as distinct, dark blue structures, measuring approximately 5µm in diameter, and often display a characteristic cup-shaped morphology, similar to coffee beans. They are frequently observed in small clusters and do not exhibit budding. Wright's stain (also known as modified Giemsa or Diff Quik stain) detected *P. jirovecii* cysts in one out of five cases tested positive for *P. jirovecii* [28].

In a study, comparing four methods for rapidly detecting *P. jirovecii* in respiratory specimens, Diff Quik stain (DQ) demonstrated a high sensitivity of 92% in sputum samples. The sensitivity for detection in BAL samples using DQ stain was 81% [8]. These findings suggest that Diff Quik stain is a reliable diagnostic tool for detecting *P. jirovecii* in immunocompromised patients, with a high sensitivity in both sputum and BAL samples. The use of this stain may be particularly useful in resource-limited settings where more advanced diagnostic techniques may not be readily available. In another study, Wright's stain detected *P. jirovecii* cysts in 9 out of 29 pulmonary aspirates. In a survey by Procop *et al*. (2004), the sensitivity and specificity of the Diff-Quik stain for detecting *P. jirovecii* were 49.2% and 99.6%, respectively [13]. Wright's stain offers several advantages for *P. jirovecii* detection, including cost-effectiveness and a relatively short 6 to 8-minute preparation time. It allows for the visualization or identification of all stages of the *P. jirovecii* life cycle, including trophozoites, sporozoites, and cysts. One notable disadvantage of this stain is its tendency to stain background material, particularly in respiratory tract specimens, such as IS samples, which can lead to false positives or difficulties in interpretation.

Our study found that Wright's stain was unsuitable for detecting *P. jirovecii* due to its limitations in differentiating the organism from background material and its indistinct morphological features. *P. jirovecii* cells can exhibit overlapping characteristics, making them

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challenging to distinguish from the background, and accurate interpretation of these specimens requires a thorough understanding of *P. jirovecii* morphology and expertise in microscopic analysis. Furthermore, the low abundance of *P. jirovecii* cells within the specimen and the potential masking effect of background material highlight the need for technical proficiency and specialized training in diagnostic microscopy.

In our study, Modified Toluidine 'O' blue staining demonstrated superiority in detecting *P. jirovecii* cysts, as it significantly enhanced their visualization and effectively eliminated background material using a sulfation reagent. This staining method offers several advantages, including its simplicity, practicality, and feasibility for routine implementation in clinical laboratory settings. Moreover, the Modified Toluidine 'O' blue stain outperformed the GMS stain in terms of sensitivity and specificity, making it a favorable alternative for routine use in clinical laboratories [14]. Therefore, we recommend the Modified Toluidine 'O' blue stain as a reliable and efficient diagnostic tool for detecting *P. jirovecii* in immunocompromised patients.

In clinically suspected PCP patients, if *P. jirovecii* is not visualized in induced sputum, it is advisable to consider obtaining a BAL sample based on clinical judgment and the availability of appropriate resources. The GMS stain has demonstrated superiority among the various staining methods employed for detecting *P. jirovecii* due to its high sensitivity and specificity in visualizing the organism's characteristic cysts and trophic forms. However, the Toluidine blue stain demonstrates comparable performance to the GMS stain, and its shorter processing time and straightforward protocol make it a viable option for routine utilization in clinical microbiology laboratories. The use of Trimethoprim+ Sulfamethoxazole for the treatment of PCP in patients with AIDS has experienced a significant increase, reflecting its effectiveness and importance in managing the disease. Consequently, detecting *P. jirovecii* has become increasingly challenging due to a substantial decrease in organism numbers. Therefore, collecting respiratory tract samples, such as IS and BAL, is recommended before initiating prophylactic treatment to ensure accurate diagnosis and appropriate management. It is essential to include PCP in the differential diagnosis of individuals with other immunocompromised conditions, given its observed frequency among renal transplant recipients.

In conclusion, our study highlights the importance of accurate diagnosis and timely management of PCP in immunocompromised patients. The Modified Toluidine blue stain is a reliable and efficient diagnostic tool for detecting *P. jirovecii*, and its use should be considered in clinical microbiology laboratories. Furthermore, collecting respiratory tract samples before initiating prophylactic treatment is crucial for ensuring accurate diagnosis and appropriate management. PCP should be included in the differential diagnosis of individuals with other immunocompromised conditions, and healthcare providers should be aware of its increasing frequency among renal transplant recipients. By implementing these recommendations, we can improve patient outcomes and reduce the burden of PCP in immunocompromised patients

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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