Journal of Tuberculosis Research, 2024, 12, 215-231
https://www.scirp.org/journal/jtr
ISSN Online: 2329-8448
ISSN Print: 2329-843X

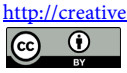
Prevalence of *Pneumocystis jirovecii* in Smear Negative Sputum Samples at the Coast General Teaching and Refferral Hospital in Kenya

Anne Saitoti[1*], Fred Wamunyokoli[1], Christine Bii[2,3]

[1]Department of Tropical Medicine and Infectious Disease, Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya
[2]Mycology Division, Center for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya
[3]Kenya Medical Research Institute, KEMRI, Nairobi, Kenya
Email: *annetesaitoti@gmail.com

How to cite this paper: Saitoti, A., Wamunyokoli, F. and Bii, C. (2024) Prevalence of *Pneumocystis jirovecii* in Smear Negative Sputum Samples at the Coast General Teaching and Refferral Hospital in Kenya. *Journal of Tuberculosis Research*, **12**, 215-231.
https://doi.org/10.4236/jtr.2024.124016

Received: February 19, 2024
Accepted: December 6, 2024
Published: December 9, 2024



Abstract

Pneumcystis jirovecii is a pathogen that causes Pneumocystis pneumonia (PCP), an infection in (HIV/ADS) and other immunocompromised patients. The rare reports of *P. jirovecii* pneumonia in sub-Saharan Africa are controversial due to the high HIV/AIDS seropositivity. The study determined the significance of *P. jirovecii* in TB smear negative retreatment patients at the Coast General Teaching Referral Hospital-Mombasa. Sputum samples were subjected to toluidine blue O(TBO) stain for microscopy and polymerase chain reaction (PCR). A total of 100 TB smear negative participants were enrolled in the study and expectorated sputum was collected. Out of the 100 patients, 63 men and 37 women. Patients aged 31 to 53 made up 62% of the patients. The patients aged 31 to 53 made up 75% of the patients (Min = 11y, Max = 85y). The median age of the patients was 42 years. Nested PCR has a prevalence of 41% (41 instances). TBO staining has a prevalence of 29% (29 instances). Detecting an additional 13 more patients than toluidine O staining technique. The sensitivity of toluidine blue O staining was 33.82%, which indicates that it correctly identified 33.82% of true positive cases compared to PCR. The specificity of toluidine blue O staining was 97.82%, indicating that it had a high ability to correctly identify true negative cases compared to PCR, suggesting that it was good at ruling out non-*P. jirovecii* cases. The study confirms that *P. jirovecii* is as a significant cause of persistent symptoms in TB patients that could be responsible for persistent symptoms despite TB treatment. We recommend fungal diagnostics in such patients before retreatment.

Keywords

Pneumocystis jirovecii, Nested PCR, *Pulmonary tuberculosis,* Smear Negative

1. Introduction

Pneumocystis jirovecii is an extracellular organism that causes pneumocystis pneumonia. It is usually found colonizing the alveolar spaces of the lungs although extra pulmonary manifestations have been reported.

Currently, the documented frequency of pneumocystis infection is increasing in Africa, with PCP detection in up to 80% of infants with pneumonia [1].

A limited number of epidemiological studies have evaluated the significance of PCP in developing countries compared to developed countries where the epidemiology and clinical picture of PCP is clearly well defined and documented [2]. Recent reports indicate an increased rate of PCP in Africa, Asia, and South America [3]. Mortality rates of 30% - 50% have been documented in several studies [4]. In persons with immunosuppressive underlying conditions, PCP carries worse prognosis and this has not changed in the past 20 years. In tuberculosis (TB) endemic parts of the world, substantial numbers of patients with pulmonary symptoms are managed as "smear negative TB patients" or treated for TB without a definitive diagnostic criterion [5] [6]. In sub-Saharan Africa, tuberculosis could be a common co-infection in persons with PCP [7]. However, the lack of access to routine PCP diagnosis is the limiting factor. The study sought to determine prevalence of *Pneumocystis jirovecii* Pneumonia (PCP) and compare specificity and sensitivity of Toluidine staining method and Polymerase Chain Reaction (PCR) in sputum of TB smear negative and retreatment patients at Coast General Hospital (CGH).

2. Materials and Methods

2.1. Study Design

The study was a cross-sectional laboratory study. Patients were recruited at the Coast General Hospital TB clinic during the study period. The laboratory procedures were done at the Mycology Division, Center for Microbiology Research, KEMRI.

2.2. Study Area

The study was carried out at the Coast General Hospital as it's the main epicenter of most patients with TB referral cases in the coast region that captures a large population of the Coast and Mombasa County.

2.3. Study Population

The patients confirmed to be TB smear negative with pulmonary tuberculosis symptoms were enrolled in the study. All consenting patients above 18 that had been diagnosed as smear negative TB and were or not being retreated for TB and attending the TB clinic at the Coast General Hospital were enrolled.

2.4. Sample Size Determination

The sample size was determined based on the 37% prevalence of PCP in an urban hospital study carried in Nairobi. Using Fischer's exact test with 95% confidence

interval;

$$n = \frac{z^2 \rho(1-\rho)}{\delta^2}$$

where: n = Sample size, z = 1.96.

p = Prevalence of PCP (37%), d = Absolute precision (0.05). Therefore

$$N = \frac{1.96^2 \times 0.1(1-0.37)}{0.05^2} = 96.8$$

We therefore recruited 100 patients from the study site.

2.5. Sampling

A total of 100 sputum samples were collected. And the quality of the sputum specimens was quantitatively accessed based on the number of squamous epithelial cells. Salivary specimens were discarded.

2.6. Toluidine Blue O Staining

The conventional staining method using TBO was used as the gold standard method for diagnosis of pneumocystis [8], the sensitivity and specificity was compared with nested PCR.

Sputum smears were made, air dried, placed in a sulphating agent, and then drained in excess cold water. The slide smears were then stained with toluidine blue o and rinsed with 95% ethanol followed by absolute ethanol and then xylene. The smears were air dried and examined at ×40 and ×100 for the presence of *Pneumocystis jirovecii* cysts or oocysts. A positive confirmation was taken if at least one cluster with characteristic cyst was detected defined as equal to or ≥ . The smears were examined for presence of oocysts and the samples with *P. jirovecii* scored as positive.

2.7. PCR Detection of *P. jirovecii*

2.7.1. DNA and Extraction

Fungal DNA was extracted from the sputum samples using the MycXtra fungal DNA extraction kit (Myconostica Ltd., Manchester, United Kingdom) according to the manufacturer's instructions.

2.7.2 PCR Methodology

Sputum samples were subjected to DNA extraction in accordance with the manufacturer's instructions. PCR was performed according to Wakefield [9].

The amplification was done using PCR primers; pAZ102E (GATGGCTGTT TCCAAGCCCA) and pAZ102H (GTGTACGTTGCAAAGTACTC), derived from the mitochondrion large subunit rRNA (mtLSUrRNA) gene.

The selected primers used avoid 5 *P. jirovecii* polymorphisms in the mtLSUrRNA and are not affected by *P. jirovecii* heterogeneity while avoiding amplification of human DNA [10] [11].

The PCR conditions were; Denaturing, annealing and extension times were run at 1 min each, at 94˚C, 55˚C and 72˚C, respectively. Amplification was for 10 and 30 cycles respectively. PCR used primer set which were repeated the latter conditions for 35 cycles using the product of the 1st PCR round as the template. The resulting amplified product of 346 bp fragment was separated on 3% gel electrophoresis for 35 minutes at 100 Volts and observed under UV Trans illuminator device. Each run of PCR was performed with a positive and negative control so as to check performance of the test.

The primers described above were used to amplify a 346 bp segment of a specific region at the Mitochondrial Large subunit gene of *P. jirovecii*. Amplification was done by adding 1 µl extracted DNA, 21 µl of nuclease free water, 0.5 µl of forward primer and 0.5 µl of reverse primer into a master mix which were lyophilized with 20 µl reaction volume containing a reaction buffer, $MgCl_2$, DNTPs and a DNA polymerase. This mixture made a total volume of 25 µl. The 2 µl was for pipetting errors. Nested PCR was performed in the first round of amplification. Denaturing, annealing and extension times were run at 1 min each, at 94˚C, 55˚C and 72˚C, respectively for 10 cycles and repeated the latter conditions for 30 cycles using the product from the 1st PCR round as the template using the same primers as the first round.

The first gel was for the positive microscopic slides that clearly had the cysts. The separation on gel agarose was done for 45 minutes and the gel was prestained with ethidium bromide.

2.8. Statistical Analysis Plan

Categorical data were tabulated and summarized using frequencies and percentages. Data was presented using bar charts and tables. To determine the association between outcome and predictor variables, a bivariate logistic regression model was fitted. The bivariate regression model was implemented in R statistical software (R Core Team, 2022).

The strength of association between *P. jirovecii* status (by nested PCR test) and demographic characteristics and risk factors (fever, night sweat and euroqol) was reported using unadjusted odds ratios, 95% confidence intervals and p-values.

Any predictor variable that would have a p-value of less than 0.2 in the bivariate model was to be entered into a multivariate regression model. A p-value of less than 0.05 was considered significant.

3. Results

3.1. Microscopy Results

Toluidine blue O stains was positive on 11% of the samples with clear stained cyst forms. Results with the TBO are shown in **Figure 1**.

The cysts were observed in clusters without budding. Very little background material is seen as the sulfation reagent digests away most of the background material but does not affect either fungal elements or *P. jirovecii*.

3.2. Molecular Results

The gel **Figure 2** was for the positive microscopic slides that clearly had the cysts and several trophozoites. The separation on gel agarose was done for 45 minutes and the electrophoresis gel was pre-stained with ethidium bromide. The above conditions were followed for the conditions and the gels pre-stained with ethidium bromide and viewed under the Trans illuminator. In all the gel runs the 100 bp ladder was used followed by the negative control and a positive control. A total of 41 samples out of the total 100 expressed the 346-band fragment on the

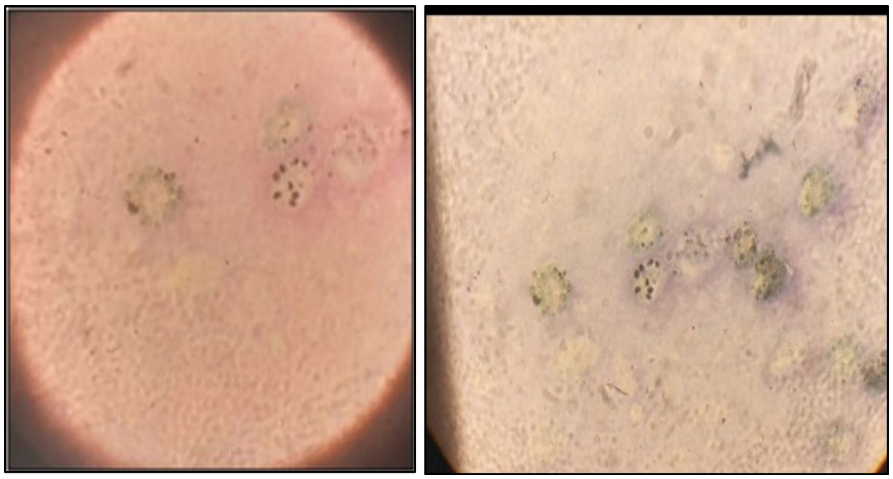


Figure 1. Toluidine blue O Stain of *P. jirovecii* ×100 magnifications In **Figures** 1, 2 the cysts approximately 5 μm in diameter are lavender with a grainy appearance, with a spherical appearance and the cell wall of the cysts are not stained. The cyst outline is distinct, and the internal region stains uniformly [12].

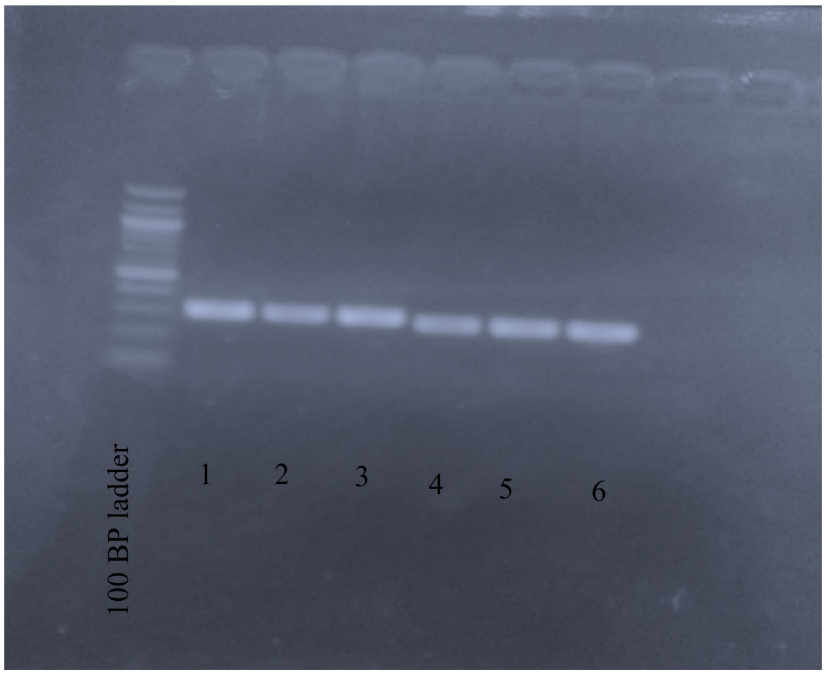


Figure 2. A gel representation of the positive *P. jirovecii* samples showing the 346 Bp gene.

agarose gel (Figure 2).

3.3. Social Demographic Data

Out of the 100 patients, 63 men and 37 women. Patients aged 31 to 53 made up 62% of the patients (Min = 11y, Max = 85y). The median age 42 years. 14 females and 27 males had the Pneumocystis Jirovecii Pneumonia. Majority of patients—35.8%—used charcoal (n = 29); 17.3% charcoal + firewood, kerosene, or gas (n = 14). 17.3% using kerosene (n = 14), 16% firewood (n = 13), and 13.6% gas (n = 11) for cooking (Table 1).

Employed were 84 (84%) and 16 (16%) unemployed among the population studied. Evidence of fungal spores' exposure through leaking or mouldy houses was reported in 28% and 26% of the population while only 9% ever smoked or were passive smokers as shown in Table 1.

Table 1. Socio-demographic characteristics of patients.

Variable	*P. jirovecii* status		
	Positive (N = 41)	Negative (N = 59)	Overall (N = 100)
Age in years			
10 - 19	6 (14.6%)	5 (8.5%)	11 (11.0%)
20 - 29	5 (12.2%)	5 (8.5%)	10 (10.0%)
30 - 39	10 (24.4%)	9 (15.3%)	19 (19.0%)
40 - 49	6 (14.6%)	17 (28.8%)	23 (23.0%)
50 - 59	7 (17.1%)	13 (22.0%)	20 (20.0%)
60+	7 (17.1%)	10 (16.9%)	17 (17.0%)
Gender			
Female	14 (34.1%)	23 (39.0%)	37 (37.0%)
Male	27 (65.9%)	36 (61.0%)	63 (63.0%)
Employment			
Employed	35 (85.4%)	49 (83.1%)	84 (84.0%)
Unemployed	6 (14.6%)	10 (16.9%)	16 (16.0%)
House Wall type			
Brick wall	21 (51.2%)	33 (55.9%)	54 (54.0%)
Concrete	8 (19.5%)	10 (16.9%)	18 (18.0%)
Makuti/Thatched/Wood	2 (4.9%)	2 (3.4%)	4 (4.0%)
Mud	10 (24.4%)	14 (23.7%)	24 (24.0%)
Floods			
No	29 (70.7%)	45 (76.3%)	74 (74.0%)

Continued

Yes	12 (29.3%)	14 (23.7%)	26 (26.0%)
TB Treatment			
Currently on Treatment	6 (14.6%)	13 (22.0%)	19 (19.0%)
Not on Treatment	35 (85.4%)	46 (78.0%)	81 (81.0%)
Fungal Growth			
No	31 (75.6%)	41 (69.5%)	72 (72.0%)
Yes	10 (24.4%)	18 (30.5%)	28 (28.0%)
Fuel			
Charcoal	16 (39.0%)	20 (33.9%)	36 (36.0%)
Charcoal/Firewood	1 (2.4%)	1 (1.7%)	2 (2.0%)
Charcoal/Gas	6 (14.6%)	6 (10.2%)	12 (12.0%)
Charcoal/Kerosene	1 (2.4%)	3 (5.1%)	4 (4.0%)
Firewood	5 (12.2%)	10 (16.9%)	15 (15.0%)
Gas	6 (14.6%)	7 (11.9%)	13 (13.0%)
Kerosene	6 (14.6%)	12 (20.3%)	18 (18.0%)
Smoking Habit			
No	39 (95.1%)	52 (88.1%)	91 (91.0%)
Yes	2 (4.9%)	7 (11.9%)	9 (9.0%)

Productive cough 87%, night sweat 71.7%, fever and chest pains 59% were most common in pneumocystis jirovecii positive individuals. Bloody cough was present in only 19.6% of the individuals while shortness of breath and fatigue were present in 54.3% and 43.5% respectively. 19% were new cases and 81% were not on any treatment despite meeting PTB clinical criteria (Table 2).

Table 2. Clinical Characteristics of patients.

Variable	*P. jirovecii status*		
	Negative (N = 59)	Positive (N = 41)	Overall (N = 100)
Fever			
No	27 (45.8%)	14 (34.1%)	41 (41.0%)
Yes	32 (54.2%)	27 (65.9%)	59 (59.0%)
Night Sweat			
No	21 (35.6%)	18 (43.9%)	39 (39.0%)
Yes	38 (64.4%)	23 (56.1%)	61 (61.0%)
Euroqol			
No	15 (25.4%)	11 (26.8%)	26 (26.0%)
Yes	44 (74.6%)	30 (73.2%)	74 (74.0%)

Table 3. Comparison of socio-demographics *P. jirovecii* Pneumonia positive patients who were Tuberculosis smear negative and being retreated at the Coast General Hospital.

Variable	Unadjusted OR	95% CI	p-value
Age in years			
10 - 19	1.71	(0.37, 8.29)	0.490
20 - 29	1.43	(0.29, 7.12)	0.656
30 - 39	1.59	(0.43, 6.13)	0.493
40 - 49	0.50	(0.13, 1.92)	0.317
50 - 59	0.77	(0.20, 2.94)	0.700
60+	Ref		
Gender			
Female	Ref		
Male	1.23	(0.54, 2.87)	0.622
Employment			
Employed	1.19	(0.40, 3.78)	0.756
Unemployed	Ref		
House Wall type			
Brick wall	Ref		
Concrete	1.26	(0.42, 3.70)	0.678
Makuti/Thatched/Wood	1.57	(0.18, 13.93)	0.663
Mud	1.12	(0.41, 2.98)	0.817
Floods			
No	Ref		
Yes	1.33	(0.53, 3.28)	0.535
TB Treatment			
Currently on Treatment	0.61	(0.20, 1.70)	0.356
Not on Treatment	Ref		
Fungal Growth			
No	Ref		
Yes	0.73	(0.29, 1.79)	0.503
Fuel			
Charcoal	Ref		
Charcoal/Firewood	1.25	(0.05, 33.29)	0.878
Charcoal/Gas	1.25	(0.33, 4.73)	0.738
Charcoal/Kerosene	0.42	(0.02, 3.61)	0.467
Firewood	0.63	(0.17, 2.14)	0.464

Continued

Gas	1.07	(0.29, 3.86)	0.915
Kerosene	0.63	(0.18, 1.99)	0.435
Smoking Habit			
No	Ref		
Yes	0.38	(0.05, 1.68)	0.245

Table 4. Risk factors associated with *P. jirovecii* Pneumonia.

Variable	Unadjusted OR	95% CI	p-value
Fever			
No	Ref		
Yes	1.63	(0.72, 3.77)	0.2468
Night Sweat			
No	Ref		
Yes	0.71	(0.31, 1.60)	0.403
Euroqol			
No	Ref		
Yes	0.931	(0.38, 2.34)	0.875

Table 5. Prevalence of *P. jirovecii* by Toluidine blue O and Nested PCR tests.

Type of Test	Prevalence *P. jirovecii* (N = 100)
Toluidine stain	
Positive	29 (29.0%)
Negative	71 (71.0%)
Nested PCR	
Positive	41 (41.0%)
Negative	59 (59.0%)

Table 6. Detection of *P. jirovecii* by Toluidine blue O and Nested PCR.

	Nested PCR		Total
	Positive	Negative	
Toluidine stain			
Positive	28	1	29
Negative	13	58	71

Table 7. Sensitivity and Specificity of Toluidine blue O as compared to Nested PCR.

	Point Estimate	Lower 95% C.I	Upper 95% C.I
Sensitivity	96.55%	82.82%	99.82
Specificity	81.69%	71.15	88.98

4. Discussion

Despite HAART medication PCP continues to be a deadly illness in AIDS patients. Previously, the prevalence of PCP among AIDS patients in Sub-Saharan Africa was unknown [13]. Recent data from Africa suggests an underreported opportunistic infection among AIDS patient. Recent research suggested that PCP may be more common in TB Patients with negative smears. The use of cotrimoxazole in HIV/AIDS patients may have impacted the prevalence of prevalence of PCP in Africa [1] [12] [13].

Out of the 100 patients, 63 men and 37 women. Patients aged 31 to 53 made up 62% of the patients (Min = 11y, Max = 85y). The median age 42 years. 14 females and 27 males had the *Pneumocystis Jirovecii* Pneumonia. Majority of patients—35.8%—used charcoal (n = 29); 17.3% charcoal + firewood, kerosene, or gas (n = 14). 17.3% using kerosene (n = 14), 16% firewood (n = 13), and 13.6% gas (n = 11) for cooking. 19 patients on TB treatment *i.e.* **Table 1**, **Table 2** and **Table 3**. Cases worsening or new cases TB treatment. 81 patients not currently on treatment but referred. Patients negative on TB diagnostic algorithms with PTB like symptoms are often considered TB relapse and retreated. As shown in **Table 4**.

Toluidine blue 0 stains also apparently stained only the cyst forms. Results with the TBO are shown in **Figure 1.** The cysts are lavender in color but are somewhat grainy in appearance; much background material is also evident. In **Figure 1** structures thought to be *P. jirovecii* cysts have a spherical appearance and the outlines of individual cysts are quite faint. The cyst forms appear as lavender structures approximately 5 μm in diameter, and many are Pneumocystis organisms have a high tropism for the lung [14] [15].

The life cycle of Pneumocystis mainly consists of 3 major life cycle stages identified by morphology; the cyst, the pre-cystic and the trophic form stages. The trophic form is mono nuclear and thin walled and usually dominates during active infection and fills the alveoli space and is variable in length 1 to 8 μm long with multiple pseudo hyphal structures which are used as an anchor to host cells [16]. The alcohol and xylene washing procedures remove excess toluidine blue O from the slide. In some other cases, *P. jirovecii* was difficult to detect because of the presence of background debris. *P. jirovecii* cysts typically occur both singly and in small clusters of closely associated organisms [17].

A total of 41 samples out of the total 100 expressed the 346-band fragment on the agarose gel. Nested PCR prevalence of 41% (41 cases) TBO staining lower prevalence of 29% (29 cases). TBO had 71 negative cases, PCR 59 negative cases as in **Table 5** and **Table 6**. Males' seemingly high prevalence susceptibility to TB could have been attributed to their poor health seeking behaviors [18].

The mitochondrial Ribosomal RNA large subunit gene is currently used as a PCR target for PCP diagnosis [9] [17]. According to a study done by Nowaseb *et al.,* 2014, *P. jirovecii* was detected in 25/475 (5.3%) patients. Seventeen patients (3.6%) tested positive for *P. Jirovecii* by qPCR and direct microscopy Eight patients (1.7%) were positive only by qPCR. None of the samples tested were positive

by direct microscopy alone [19].

Mt LSU-rRNA, is one of the highly sensitive and specific genetic regions because they are multiple copies of genomes in it [19]. The Mt LSU-rRNA gene is involved in basic metabolic functions and is considered to be a highly informative locus. This is also the locus used most widely for PCR-based detection of *P. jirovecii*, as it is present in a large number of copies [20].

Nested PCR additional 12 patients than TBO technique-high sensitivity, TBO had acceptable sensitivity & very high specificity (**Table 6**). In developing countries rates of PJP are lower, possibly due to poor survival, under diagnosis, prior infection with tuberculosis and other organisms [21]-[23].

However, locally no studies have been done to determine the significance of co-infection of *Pneumocystis* pneumonia with tuberculosis [24] [25].

Given that most tuberculosis patients are co-infected with HIV/AIDS and are severely immunocompromised [26] [27], there is a possibility that *Pneumocystis* pneumonia is a significant co-pathogen in this group of patients [28] [29].

Pneumocystis pneumonia has been shown to be a co-infecting pathogen with other respiratory pathogens with a prevalence of [30]-[32].

However the majority, 89% of this study specimens were negative for *P. jirovecii*, These findings highlight the neccesity and the importance of fungal diagnosis in high risk population especially those with pulmonary TB *[33]* [34]. According to this study chi-squire test, there was no significance of co-infection *Pneumocystis jirovecii* with *Mycobacterium tuberculosis* with p-value of 0.86 [35] [36]. In order to evaluate the sensitivity and specificity of Nested PCR and Toluidine Blue O staining method in identifying *P. jirovecii* in the sputum of patients [37] [38] with tuberculosis smear negative and retreatment cases with the nested PCR method serving as the gold standard as shown in **Table 7**.

The sensitivity of toluidine blue O staining is 33.82%, which indicates that it correctly identifies 33.82% The specificity of toluidine blue O staining is 97.82%, indicating that it has a high ability to correctly identify true negative cases compared to PCR, suggesting that it is good at ruling out non-*P. jirovecii* cases.

The sensitivity of toluidine blue O staining is 33.82%, which indicates that it correctly identifies 33.82% of true positive cases compared to PCR, missing a significant proportion of true positive cases. The specificity of toluidine blue O staining is 97.82%, indicating that it has a high ability to correctly identify true negative cases compared to PCR, suggesting that it is good at ruling out non-*P. jirovecii* cases [39]-[42].

The Positive Predictive Value (PPV) of toluidine blue O staining is 95.83%, indicating that when it gives a positive result, there is a 95.83% chance that the patient actually has *P. jirovecii* according to PCR. This suggests that a positive result with toluidine blue O staining is highly likely to be accurate (**Table 7**).

The Negative Predictive Value (NPV) of toluidine blue O staining is 78.95%, indicating that when it gives a negative result, there is a 78.95% chance that the patient truly does not have *P. jirovecii* according to PCR (**Table 7**). A negative result with toluidine blue O staining, however, should be interpreted with caution,

as it has a relatively higher rate of false negatives. The accuracy of toluidine blue O staining is 83.95%, which indicates the overall proportion of correct results (true positives + true negatives) compared to PCR [32].

A positive TBO stain usually indicated PCP, so this may be considered the minimum frequency of infection (3.6%) [43] [44]. Simultaneous co-contamination with *M. tuberculosis* and *P. jirovecii* isn't always thought to be common, which may explain why the prevalence of *P. jirovecii* appeared to be low in regions with high *M. tuberculosis* burdens [45] [46]. The presence of PCP might also suggest colonization of those patients; however, it became more likely that Candida became a co-present oral pathogen in sufferers with PCP, because oral candidiasis is commonplace in these patients over the last decade, research have indicated that PCP can be more common in TB smear-negative sufferers [47] [48]. The doubtful image of PCP incidence in Africa can be because of empirical remedy and prophylaxis the usage of cotrimoxazole in HIV/AIDS patients [49].

5. Conclusions

Pneumonia is a significant economic burden on the healthcare system. In the developing world rates of PCP are lower, possibly due to poor survival, under diagnosis and prior infection with tuberculosis and other organisms [22].

Sensitive and specific diagnostic tools are warranted in resource constrained setting and PCR presents a potential rapid sensitive diagnostic tool. Considering cost simplicity and efficacy we recommend TBO as the most practical diagnostic test for use in resource-constrained setting.

The data obtained from this study is an indication of the coinfection of fungi and pulmonary tuberculosis. The study signifies the need for an evaluation of the recurring pulmonary tuberculosis and prudent antifungal treatment based on the culture results rather than broad-spectrum antibiotics for cure. Induced sputum is more cost-effective; however, it is also of limited use because it requires staff training, patient preparation and dedicated rooms. A few studies have compared the value of induced sputum with expectorated sputum and they concluded that the difference in yield was negligible. The current study demonstrated that expectorated sputum can be used in resource-limited settings in sub-Saharan Africa for the routine diagnosis of PCP.

6. Limitation of Study

Pneumocystis jirovecii is fastidious and thus *in vitro* culture is difficult. As Pneumocystis remains a non-cultivable microorganism, traditional diagnostic methods rely on microscopic observations of the pathogen in respiratory specimen which is inherently non-specific.

7. Recommendation

Smear negative patients presenting with clinical symptoms of Tuberculosis the long run should undergo mycological testing before treatment. This necessitates

investment in technical and infrastructure capabilities for the diagnosis of fungal illness such as *Pneumocystis jirovecii*.

Acknowledgements

This study was funded by the Kenya Medical Research Institute. I acknowledge my supervisors for their essential evaluation of my work and their consistent encouragement. Secondly, I acknowledge the Centre for Microbiology Research (CMR), KEMRI, for the supply of laboratory area and the work force. I additionally acknowledge the Coast general hospital, Mombasa for their help in acquiring samples from their hospital. Ultimately, my colleagues, friends and family for their consistent support.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Abouya, Y.L., Beaumel, A., Lucas, S., Dago-Akribi, A., Coulibaly, G., N'dhatz, M., *et al.* (1992) *Pneumocystis carinii* Pneumonia: An Uncommon Cause of Death in African Patients with Acquired Immunodeficiency Syndrome. *American Review of Respiratory Disease*, **145**, 617-620. https://doi.org/10.1164/ajrccm/145.3.617

[2] Apostolopoulou, A. and Fishman, J.A. (2022) The Pathogenesis and Diagnosis of *Pneumocystis jiroveci* Pneumonia. *Journal of Fungi*, **8**, Article 1167. https://doi.org/10.3390/jof8111167

[3] Bartlett, M.S. and Smith, J.W. (1991) *Pneumocystis carinii*, an Opportunist in Immunocompromised Patients. *Clinical Microbiology Reviews*, **4**, 137-149. https://doi.org/10.1128/cmr.4.2.137

[4] Bateman, M., Oladele, R. and Kolls, J.K. (2020) Diagnosing *Pneumocystis jirovecii* Pneumonia: A Review of Current Methods and Novel Approaches. *Medical Mycology*, **58**, 1015-1028. https://doi.org/10.1093/mmy/myaa024

[5] Bii, C.C., Kose, J., Taguchi, H., Amukoye, E., Ouko, T.T., Muita, L.C., *et al.* (2006) *Pneumocystis jirovecii* and Microbiological Findings in Children with Severe Pneumonia in Nairobi, Kenya. *The International Journal of Tuberculosis and Lung Disease*, **10**, 1286-1291.

[6] Calderonsandubete, E. (2002) Historical Perspective on *Pneumocystis carinii* Infection. *Protist*, **153**, 303-310. https://doi.org/10.1078/1434-4610-00107

[7] Carmona, E.M. and Limper, A.H. (2010) Update on the Diagnosis and Treatment of *Pneumocystis* Pneumonia. *Therapeutic Advances in Respiratory Disease*, **5**, 41-59. https://doi.org/10.1177/1753465810380102

[8] Chakaya, J.M., Bii, C., Ng'ang'a, L., Amukoye, E., Ouko, T., Muita, L., *et al.* (2004) *Pneumocystis carinii* Pneumonia in HIV/AIDS Patients at an Urban District Hospital in Kenya. *East African Medical Journal*, **80**, 30-35. https://doi.org/10.4314/eamj.v80i1.8663

[9] Chawla, K., Martena, S., Gurung, B., Mukhopadhyay, C., Varghese, G. and Bairy, I. (2011) Role of PCR for Diagnosing *Pneumocystis jirovecii* Pneumonia in HIV-Infected Individuals in a Tertiary Care Hospital in India. *Indian Journal of Pathology and Microbiology*, **54**, 326-329. https://doi.org/10.4103/0377-4929.81624

[10] Cushion, M.T. (2010) Are Members of the Fungal Genus *Pneumocystis* (a) Commensals; (b) Opportunists; (c) Pathogens; or (d) All of the Above? *PLOS Pathogens*, **6**, e1001009. https://doi.org/10.1371/journal.ppat.1001009

[11] Cushion, M.T., Linke, M.J., Ashbaugh, A., Sesterhenn, T., Collins, M.S., Lynch, K., *et al.* (2010) Echinocandin Treatment of *Pneumocystis* Pneumonia in Rodent Models Depletes Cysts Leaving Trophic Burdens That Cannot Transmit the Infection. *PLOS ONE*, **5**, e8524. https://doi.org/10.1371/journal.pone.0008524

[12] De Armas Rodríguez, Y., Wissmann, G., Müller, A.L., Pederiva, M.A., Brum, M.C., Brackmann, R.L., *et al.* (2011) *Pneumocystis jirovecii* Pneumonia in Developing Countries. *Parasite*, **18**, 219-228. https://doi.org/10.1051/parasite/2011183219

[13] Eddens, T. and Kolls, J.K. (2014) Pathological and Protective Immunity to *Pneumocystis* Infection. *Seminars in Immunopathology*, **37**, 153-162. https://doi.org/10.1007/s00281-014-0459-z

[14] Ewig, S., Bauer, T., Schneider, C., Pickenhain, A., Pizzulli, L., Loos, U., *et al.* (1995) Clinical Characteristics and Outcome of *Pneumocystis carinii* Pneumonia in HIV-Infected and Otherwise Immunosuppressed Patients. *European Respiratory Journal*, **8**, 1548-1553. https://doi.org/10.1183/09031936.95.08091548

[15] Harris, J.R., Marston, B.J., Sangrujee, N., DuPlessis, D. and Park, B. (2011) Cost-Effectiveness Analysis of Diagnostic Options for *Pneumocystis* Pneumonia (PCP). *PLOS ONE*, **6**, e23158. https://doi.org/10.1371/journal.pone.0023158

[16] Helweg-Larsen, J., Jensen, J.S., Dohn, B., Benfield, T.L. and Lundgren, B. (2002) Detection of *Pneumocystis* DNA in Samples from Patients Suspected of Bacterial Pneumonia—A Case-Control Study. *BMC Infectious Diseases*, **2**, Article No. 28. https://doi.org/10.1186/1471-2334-2-28

[17] Homayouni, M.M., Behniafar, H., Mehbod, A.S.A., Mohammad-Sadeghi, M. and Maleki, B. (2017) Prevalence of *Pneumocystis jirovecii* among Immunocompromised Patients in Hospitals of Tehran City, Iran. *Journal of Parasitic Diseases*, **41**, 850-853. https://doi.org/10.1007/s12639-017-0901-y

[18] Huang, L. and Crothers, K. (2009) HIV-Associated Opportunistic Pneumonias. *Respirology*, **14**, 474-485. https://doi.org/10.1111/j.1440-1843.2009.01534.x

[19] Huang, L., Morris, A., Limper, A.H. and Beck, J.M. (2006) An Official ATS Workshop Summary: Recent Advances and Future Directions in *Pneumocystis* Pneumonia (PCP). *Proceedings of the American Thoracic Society*, **3**, 655-664. https://doi.org/10.1513/pats.200602-015ms

[20] Kovacs, J.A. and Masur, H. (2009) Evolving Health Effects of *Pneumocystis*: One Hundred Years of Progress in Diagnosis and Treatment. *JAMA*, **301**, 2578-2585. https://doi.org/10.1001/jama.2009.880

[21] Minor, O.L., Germani, Y., Chartier, L., Lan, N.H., Lan, N.T.P., Duc, N.H., *et al.* (2008) Predictors of Pneumocystosis or Tuberculosis in HIV-Infected Asian Patients with AFB Smear-Negative Sputum Pneumonia. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, **48**, 620-627. https://doi.org/10.1097/qai.0b013e31817efb3c

[22] Lowe, D.M., Rangaka, M.X., Gordon, F., James, C.D. and Miller, R.F. (2013) *Pneumocystis jirovecii* Pneumonia in Tropical and Low and Middle Income Countries: A Systematic Review and Meta-Regression. *PLOS ONE*, **8**, e69969. https://doi.org/10.1371/journal.pone.0069969

[23] Lu, Y., Ling, G., Qiang, C., Ming, Q., Wu, C., Wang, K., *et al.* (2011) PCR Diagnosis of *Pneumocystis* Pneumonia: A Bivariate Meta-Analysis. *Journal of Clinical Microbiology*, **49**, 4361-4363. https://doi.org/10.1128/jcm.06066-11

[24] Mohamed, A., Obanda, B.A., Njeri, H.K., Loroyokie, S.N., Mashedi, O.M., Ouko, T.T., *et al.* (2022) Serological Evidence of Chronic Pulmonary Aspergillosis in Tuberculosis Patients in Kenya. *BMC Infectious Diseases*, **22**, Article No. 798. https://doi.org/10.1186/s12879-022-07782-9

[25] Jarboui, M.A., Mseddi, F., Sellami, H., Sellami, A., Makni, F. and Ayadi, A. (2013) Genetic Diversity of *Pneumocystis Jirovecii* Strains Based on Sequence Variation of Different DNA Region. *Medical Mycology*, **51**, 561-567. https://doi.org/10.3109/13693786.2012.744879

[26] Morris, A. and Norris, K.A. (2012) Colonization by *Pneumocystis jirovecii* and Its Role in Disease. *Clinical Microbiology Reviews*, **25**, 297-317. https://doi.org/10.1128/cmr.00013-12

[27] Morris, A., Lundgren, J.D., Masur, H., Walzer, P.D., Hanson, D.L., Frederick, T., *et al.* (2004) Current Epidemiology of *Pneumocystis* Pneumonia. *Emerging Infectious Diseases*, **10**, 1713-1720. https://doi.org/10.3201/eid1010.030985

[28] Murray J.F. (2005) Pulmonary Complications of HIV-1 Infection among Adults Living in Sub-Saharan Africa. *The International Journal of Tuberculosis and Lung Disease*, **9**, 826-835.

[29] Mwaura, E.N., Matiru, V. and Bii, C. (2013) Mycological Findings of Sputum Samples from Pulmonary Tuberculosis Patients Attending TB Clinic in Nairobi, Kenya. *Virology & Mycology*, **2**, Article 1000119. https://doi.org/10.4172/2161-0517.1000119

[30] Nowaseb, V., Gaeb, E., Fraczek, M.G., Richardson, M.D. and Denning, D.W. (2014) Frequency of *Pneumocystis jirovecii* in Sputum from HIV and TB Patients in Namibia. *The Journal of Infection in Developing Countries*, **8**, 349-357. https://doi.org/10.3855/jidc.3864

[31] Onishi, A., Sugiyama, D., Kogata, Y., Saegusa, J., Sugimoto, T., Kawano, S., *et al.* (2012) Diagnostic Accuracy of Serum 1,3-β-D-Glucan for *Pneumocystis jiroveci* Pneumonia, Invasive Candidiasis, and Invasive Aspergillosis: Systematic Review and Meta-Analysis. *Journal of Clinical Microbiology*, **50**, 7-15. https://doi.org/10.1128/jcm.05267-11

[32] Patel, N. and Koziel, H. (2004) *Pneumocystis jiroveci* Pneumonia in Adult Patients with AIDS: Treatment Strategies and Emerging Challenges to Antimicrobial Therapy. *Treatments in Respiratory Medicine*, **3**, 381-397. https://doi.org/10.2165/00151829-200403060-00005

[33] Rath, P. and Steinmann, J. (2014) Update on Diagnosis of *Pneumocystis* Pulmonary Infections. *Current Fungal Infection Reports*, **8**, 227-234. https://doi.org/10.1007/s12281-014-0188-8

[34] Reid, A.B., Chen, S.C.-A. and Worth, L.J. (2011) *Pneumocystis jirovecii* Pneumonia in Non-HIV-Infected Patients: New Risks and Diagnostic Tools. *Current Opinion in Infectious Diseases*, **24**, 534-544. https://doi.org/10.1097/qco.0b013e32834cac17

[35] Roux, A., Canet, E., Valade, S., Gangneux-Robert, F., Hamane, S., Lafabrie, A., *et al.* (2014) *Pneumocystis jirovecii* Pneumonia in Patients with or without AIDS, France. *Emerging Infectious Diseases*, **20**, 1490-1497. https://doi.org/10.3201/eid2009.131668

[36] Roux, A., Gonzalez, F., Roux, M., Mehrad, M., Menotti, J., Zahar, J.-R., *et al.* (2014) Update on Pulmonary *Pneumocystis jirovecii* Infection in Non-HIV Patients. *Médecine et Maladies Infectieuses*, **44**, 185-198. https://doi.org/10.1016/j.medmal.2014.01.007

[37] Russian, D.A. and Levine, S.J. (2001) *Pneumocystis carinii* Pneumonia in Patients

without HIV Infection. *The American Journal of the Medical Sciences*, **321**, 56-65. https://doi.org/10.1097/00000441-200101000-00009

[38] Masur, H., Kovacs, J. and Siegel, M. (2016) *Pneumocystis jirovecii* Pneumonia in Human Immunodeficiency Virus Infection. *Seminars in Respiratory and Critical Care Medicine*, **37**, 243-256. https://doi.org/10.1055/s-0036-1579556

[39] Slogrove, A.L., Cotton, M.F. and Esser, M.M. (2009) Severe Infections in HIV-Exposed Uninfected Infants: Clinical Evidence of Immunodeficiency. *Journal of Tropical Pediatrics*, **56**, 75-81. https://doi.org/10.1093/tropej/fmp057

[40] Thomas, C.F. and Limper, A.H. (2004) *Pneumocystis* Pneumonia. *New England Journal of Medicine*, **350**, 2487-2498. https://doi.org/10.1056/nejmra032588

[41] Thomas, C.F. and Limper, A.H. (2007) Current Insights into the Biology and Pathogenesis of *Pneumocystis* Pneumonia. *Nature Reviews Microbiology*, **5**, 298-308. https://doi.org/10.1038/nrmicro1621

[42] Luísa Tomás, A. and Matos, O. (2018) *Pneumocystis jirovecii* Pneumonia: Current Advances in Laboratory Diagnosis. *OBM Genetics*, **2**, Article 49. https://doi.org/10.21926/obm.genet.1804049

[43] Toper, C., Rivaud, E., Daniel, C., Cerf, C., Parquin, F., Catherinot, É., *et al.* (2011) Pneumocystose pulmonaire chez des patients immunodéprimés non infectés par le VIH: Étude de 41 cas. *Revue de Pneumologie Clinique*, **67**, 191-198. https://doi.org/10.1016/j.pneumo.2011.06.001

[44] Tufa, T.B. and Denning, D.W. (2019) The Burden of Fungal Infections in Ethiopia. *Journal of Fungi*, **5**, Article 109. https://doi.org/10.3390/jof5040109

[45] Vargas, S.L., A. Ponce, C., Gigliotti, F., Ulloa, A.V., Prieto, S., Muñoz, M.P., *et al.* (2000) Transmission of *Pneumocystis carinii* DNA from a Patient with *P. Carinii* Pneumonia to Immunocompetent Contact Health Care Workers. *Journal of Clinical Microbiology*, **38**, 1536-1538. https://doi.org/10.1128/jcm.38.4.1536-1538.2000

[46] Vray, M., Germani, Y., Chan, S., Duc, N.H., Sar, B., Sarr, F.D., *et al.* (2008) Clinical Features and Etiology of Pneumonia in Acid-Fast Bacillus Sputum Smear-Negative HIV-Infected Patients Hospitalized in Asia and Africa. *AIDS*, **22**, 1323-1332. https://doi.org/10.1097/qad.0b013e3282fdf8bf

[47] Wakefield, A.E. (1996) DNA Sequences Identical to *Pneumocystis carinii* f. sp. *carinii* and *Pneumocystis carinii* f. sp. Hominis in Samples of Air SPORA. *Journal of Clinical Microbiology*, **34**, 1754-1759. https://doi.org/10.1128/jcm.34.7.1754-1759.1996

[48] Worodria, W., Okot-Nwang, M., Yoo, S.D. and Aisu, T. (2003) Causes of Lower Respiratory Infection in HIV-Infected Ugandan Adults Who Are Sputum AFB Smear-Negative. *The International Journal of Tuberculosis and Lung Disease*, **7**, 117-123.

[49] Zakrzewska, M., Roszkowska, R., Zakrzewski, M. and Maciorkow-Ska, E. (2019) *Pneumocystis* Pneumonia: Still a Serious Disease in Children. *Journal of Mother and Child*, **23**, 159-162. https://doi.org/10.34763/devperiodmed.20192303.159162

Abbreviations and Acronyms

TB	Tuberculosis
KEMRI	Kenya Medical Research Institute
PCP/PJP	*Pneumocystis jirovecii* Pneumonia
SERU	Scientific and Ethics Review Unit
Retreatment Cases	Any individual with a history of treatment for tuberculosis exhibiting clinical symptoms consistent with pulmonary tuberculosis.
PCR	Polymerase Chain Reaction
CGH	Coast General Teaching and Referral Hospital