Biomedicine and Chemical Sciences 1(4) (2022) 225-233

29-BCS-1007-235

Content lists available at:
https://journals.irapa.org/index.php/BCS/issue/view/15

Biomedicine and Chemical Sciences

Journal homepage: https://journals.irapa.org/index.php/BCS

Conventional PCR versus Culture Method to Detect Common Fungal Pathogens in Patients with Respiratory Diseases

Hadeel Jamal Rashied[a]*, Azhar A. Al-Attraqchi[b], Amar Kasim Muhmmed[c]

[a] *Department of Teaching Laboratory, AL-Emamain AL-Khadhymian Teaching Hospital, Baghdad – Iraq*
[b] *Department of Microbiology/ College of Medicine/ Al-Nahrain University, Baghdad – Iraq*
[c] *Department of Medicine Baghdad Teaching hospital, Medical City Baghdad – Iraq*

ARTICLE INFO

Article history:
Received on: March 31, 2022
Revised on: June 12, 2022
Accepted on: June 12, 2022
Published on: October 01, 2022

Keywords:
Conventional methods
Fungal infection
ITS
PCR technique
Respiratory disease

ABSTRACT

The study aimed to assess the frequency of invasive fungal infection in patients with respiratory diseases by conventional and molecular methods. This study included 117 Broncho alveolar lavage (BAL) samples were collected from patients with respiratory disease (79 male and 38 female) with ages ranged between (20-80) years, who attended Medicine Baghdad Teaching hospital and AL-Emamain AL-Khadhymian Medical City, during the period from September 2019 to April 2020. The results in PCR versus culture methods in this study showed that out of 117 samples of fungal infections 30(25.6 %) were detected by culture method, while the 24(20.5%) samples were detected by PCR technique**,** the most commonly diagnosed pathogenic fungi is *Candida* spp. followed by *Aspergillus* spp. By considering the culture method as a gold standard against the PCR technique, the results show that the sensitivity and specificity of PCR were (86.6%) and (100%) respectively.

1. Introduction

[1]Respiratory fungal infection is a severe clinical problem, especially in patients with compromised immune functions. Lower respiratory tract infections (LRTIs) include acute bronchitis, bronchiolitis and pneumonia; the symptom can range from mild to severe (Cao et al., 2013). Fungal pneumonia is caused by one or endemic or opportunistic fungi, the infection occurs in both immune-competent and immune-compromised patients. During recent decades, the incidence of opportunistic mycosis has greatly increased especially in immune-compromised such as patients receiving immune-suppressive therapy, that undergoing bone marrow transplantation or a solid-organ transplant, diabetes mellitus and cancer patients (José et al., 2020).

Candida spp. *Aspergillus* spp., *Cryptococcus* spp. and *Mucor* spp. are major pulmonary fungal pathogens that are able to result in life-threatening invasive diseases (Mikulska et al., 2019). BAL fluid was the most frequently tested material in pulmonary disease. Culture is still a gold standard for isolated fungi, more sensitive than direct microscopy, unfortunately, many fungal organisms may require several days to a couple of weeks to yield and identify. It is time-consuming (Arvanitis et al., 2014) while direct microscopic examination of candida in (BAL) fluid was more sensitive than culture, but utilization is limited as it requires expertise for interpretations and is distinguished poorly among fungi with similar morphology (Louisiana Office of Public Health, 2010).

Internal Transcribed Spacer (ITS) region of nuclear DNA (rDNA) has become the most sequenced region to identify fungal taxonomy at a species level, and even within species. ITS region is a highly polymorphic non-coding region with enough taxonomic units (Fajarningsih, 2016) is the universal fungal barcode sequence. Includes ITS1, ITS2, ITS3 and ITS4 regions, separated by the 5.8S gene, and are situated between the 18S (SSU) and 28S (LSU) genes

***Corresponding author:** Hadeel Jamal Rashied, Department of Teaching Laboratory, AL-Emamain AL-Khadhymian Teaching Hospital, Baghdad – Iraq

E-mail: **hadeeljamal654@gmail.com**

How to cite:

Rashied, H. J., Al-Attraqchi, A. A., & Muhmmed, A. K. (2022). Conventional PCR versus Culture Method to Detect Common Fungal Pathogens in Patients with Respiratory Diseases. *Biomedicine and Chemical Sciences, 1*(4), 225–233.

DOI: **https://doi.org/10.48112/bcs.v1i4.235**

(Bellemain, et al., 2010). The ITS gene was selected for several reasons:

- Its universal conserved regions, make it possible to obtain the PCR products from most of the fungi
- The large number of sequences in GenBank makes similarity searches convenient and facilitates the start of extensive laboratory screenings
- (ITS) region evolves relatively slowly and is thus more suitable for finding consensus conserved regions within a group of fungi

Finally, the repetitive nature, over 100 copies of which usually exist per fungal genome makes the region an appealing target for sequencing environmental substrates where the quantity of DNA present is low (Bellemain et al., 2010).

The development of molecular approaches has greatly facilitated the identification of fungal pathogens, PCR is one of the most widely used molecular methods in fungal diagnosis, and has the potential to achieve rapid, sensitive, specific detection and identification of fungi from the direct methods or culture (Lengerova et al., 2014). The aims of this study were to assess the frequency of fungal infections as causative agents of different respiratory infections and Carrying out a comparison between conventional (direct &culture) and molecular methods for the detection of pathogenic fungi among patients.

2. Materials and Methods

This study included 117 (BAL) samples were been collected from patients with respiratory disease (79 male, 38 female) with ages ranged between (20-80) years, who attended Medical Baghdad Teaching Hospital and AL-Emamain AL-Khadhymian, during the period from September 2019 to April 2020.

Twenty milliliters of BAL samples were collected. Ten milliliters samples for conventional works (direct and culture) were centrifuged and the sediment was inoculated on Sabouraud's agar at 37˚C for 24 hours for candida and at (28 °C±2°C) for (2-5) days for other fungi. The direct examination was done by using Lacto phenol cotton blue stain for general fungal identification. Other ten ml of samples were used for molecular methods, DNA was extracted **(**ABIOpure™ Total DNA Extraction Kit) from BAL lavage, then all primers were supplied in a lyophilized form. (ITS1, ITS4 gene ribosomal DNA region, Aspergillus.*BGT1*, Candida *CaVPS13*, Cryptococcus *PLB1*, and Mucor *MucL*, *MR1*), were dissolved in a nuclease free water to give a final concentration of 100pmol/µl as a stock solution conventional polymerase chain reaction (PCR) method was used, PCR products were run on gel electrophoresis.

The first run of conventional (PCR) was used with the help of primer internal transcribed spacer (ITS1and ITS4). The temperature and time of PCR program were optimized by using gradient PCR. The negative control contained all materials with the substitute of distilled water instead of template DNA. Reaction tubes were placed into the thermo cycler was performed in a total volume of 20 µl, the PCR component calculation for *ITS*, CaVPS13*, BGT1, MucL1 and PLB,* including 20µl volumes containing Master Mix (10X), 2µl from Primers stock and 4µl of DNA template finally with 4µl Nuclease free water. The program conditions of amplification ITS gene include, initial denaturation at 95°C for 5 min. Followed by denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension was given at 72°C for 30 s, final extension of 7 min at 72°C.

The second run conventional (PCR) was used for the detection of the gene specific for *Candida.* spp*, Aspergillus* spp.*, Mucor* spp. *and Cryptococcus neoformans*. The program condition for each gene in this study includes initial denaturation at 95°C for 5 min. followed by denaturation at 95°C for 30 s, annealing at (50°C, 55°C, 60°C, 61°C for 30 s, and extension was given at 72°C for 30 s, final extension of 7 min at 72°C. The statistical analysis of this prospective study performed with the statistical package for social sciences (SPSS) 21.0 software and Microsoft Excel 2013. Numerical data were described as mean and standard deviation. While, categorical data were described as count and percentage.

Sensitivity, specificity, positive predictive value and negative predictive value were calculated. The lower level of accepted statistical significant difference is equal or bellow to 0.05.

3. Results and Discussion

3.1. Patients Descriptive Data

This study involved one hundred seventeen patients with respiratory diseases. The patients' age ranged from (20-80) years with mean age (58) years. The highest percentage of fungal infection was seen among (34%) of patients in the age group (61-70) years old, while the lowest one occurred only (3%) of patients in the age group (31-40) years old. Regarding sex features, the distribution of infection according to age intervals was a significant association between the age group and sex seen when P = 0.055, as shown in Figure 1.

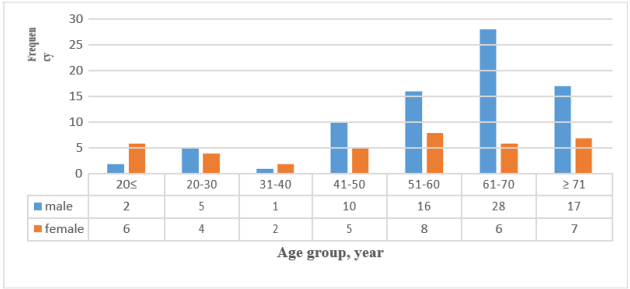

Fig. 1. Sex distribution according to age group among infected patients

The current study shows that the highest fungal infection was seen among old patients, this is in agreement with (Njovu et al., 2021) in Uganda who saw that the highest age group was 60>, while it disagrees with the study by (Sani et al., 2020) in Nigeria. Studies conducted by (Ogba et al. 2013) and (Jha et al., 2013) recorded that the highest age group developed fungal infected was (25–40) years. The most probable reason may be due to the high activity of patients in this age bracket. The participants with pulmonary fungal pathogens were mostly those ≥50 years compared to patients within lower age groups. The older adults become more susceptible to diseases due to predisposing factors as increased exposure to micro-organisms, outdoor activities, age-related physiological changes such as degeneration of various organs (arthritis, diabetes mellitus, heart disease), receiving aggressive regimens of chemotherapy, taking

immunosuppressive drugs for nonmalignant diseases, all these causes decrease immune response. In addition to that, older individuals are more likely to travel, which put them at risk of exposure to endemic mycoses that can make these age groups more prone to fungal colonization and/or infection (Baddley et al., 2011).

Regarding chronic disease and its relation with fungal infection and sex factor, in case of DM (diabetes mellitus) it was formed the highest percentage 40(50.6%) in males, while11 (28.9%) in females, add in cancer case there was 3(3.8%) in males and 6 (15.7%) in females as shown in Table 1.

Table 1
Distribution of Sex according to disease

Disease	sex		Total	%
	Female (N=38) %	Male (N=79) %		
Cancer	6 15.7	3 3.8	9	7.6
DM	11 28.9	40 50.6	51	43.5

DM = diabetes Mellitus

Regarding underlying diseases in the current study, the rate of diabetes mellitus (DM) was (43.5%) represented by 51 patients in males and females with fungal infection which is in agreement with a study conducted in Nepal by (Saud, et al. 2020) who reported the prevalence of fungus was (34.0%) in diabetics and (4.7%) in non-diabetics. It means the prevalence of fungal colonization in diabetics is eight times higher than that in no diabetic participants. In addition, another study in Portugal by (Rodrigues, et al. 2019) proved that DM patients have an increased susceptibility to fungal infections, in cases of uncontrolled hyperglycemia. A study in China by (Lao et al., 2019) reported invasive fungal disease was diagnosed in 120 patients with diabetes mellitus, the yeast infection was (46.7%) and mold infection was (40.8%) while a total of (12.7%) patients had mixed fungal infection. Hyperglycemic state and acidosis cause promote fungal growth and immune dysfunction which lead to local and systemic infection due to overgrowth of microflora which causes an opportunistic infection (Mandell, *et al.*, 2005). In the case of cancer, there were 9 (7.6%) in both males and females; Cancer treatments such as chemotherapy and radiation can naturally lower the body's immune system, which can give the opportunity for fungal infections to occur (Puebla, 2012). In case of smoking, out of the 117 there were 54 (90 %) smoker in males while 6 (10%) in female as shown in Figure 2.

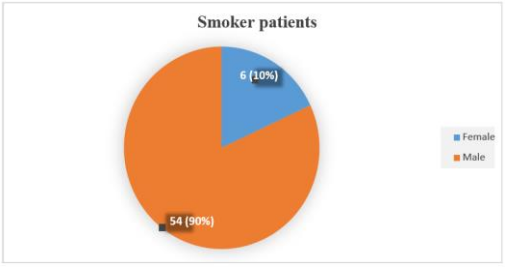

Fig. 2. Frequency of smokers regarding sex factor

These results are concomitant with most studies (Akram et al., 2018) in Bosnia and Herzegovina, patients with the presence of oral candida were smokers in (82.5%) cases, while patients without candida were smokers in (44%) cases, (75 male and 65 female). Smoking has an influence on oral colonization with *Candida* spp. Cigarette smoking promotes adhesion and biofilm formation, which may explain the increased persistence of the pathogen in smokers, the adhesion of yeast to host tissue is the initial phase of a potential infection that enables fungi to survive inside the host and eventually colonize host tissues.

3.2. Laboratory Diagnosis: Conventional Methods

Out of 117 BAL samples, *Candida* spp. was seen in 28 (23.9%), distributed as in males 17 (60.8%) while in females 11(39.2%) upon direct microscopic examination. By Lacto phenol cotton blue, while regarding the culture methods, out of the 117 BAL samples tested 30 (25.7%) samples belonged to fungus-infected patients, while non-fungus-infected patients were 87 (74.3%) samples, there are different associations between infected and non-infected patients. This result comes close to this done by (Jahromi et al., 2020) in Iran, who proved the prevalence of fungi in respiratory infection was (36.6%), (Rafat et al., 2020) in Iran also reported the prevalence was (35.6%) and (Roohani et al., 2018) in India reported the prevalence was16 (32.0%). While it disagreed with the result obtained by (Ahmed et al., 2019) in Egypt who showed that the prevalence of mycosis was 40 (66.6%). In the current result, the percentage of fungal respiratory infection was considered low due to the limitations of the study which was the small number of cases collected, thus our findings may not be representative of the entire population of Baghdad; other factors were the relatively short period during which patients were selected from different respiratory units. Regarding the genus of isolated fungi on Sabouraud dextrose agar, it was seen that out of 30 positive samples, twenty (66.6%) samples were *Candida* spp., five (16.6%) samples were *Aspergillus* spp., 4 (13.3%) samples were *Cryptococcus* spp., and only one (3.3%) sample as *Mucor* spp. as shown in Figure 3:

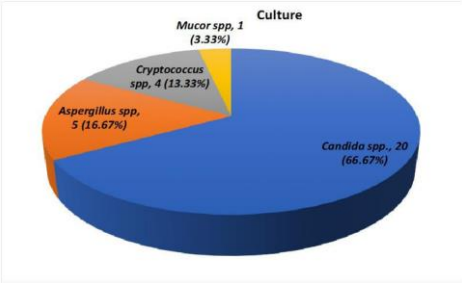

Fig. 3. Frequency of fungal among patients with respiratory disease detected by culture methods

In the current study, the higher isolated genus was *Candida* spp.as 20 (66.6%), followed by five (16.6%) samples were *Aspergillus* spp.; these findings agreed with the study done by (Chadana, et al. 2019) in India and a study by (Pendleton et al., 2017) in the USA, revealed that candida is the most commonly isolated fungal genus detected in respiratory specimens . However, the findings disagree with a study by (Bezdicek et al., 2016) in the USA. Thhe study reported that *Aspergillus* spp. are still the most common pathogens, followed by *Candida* spp., this could have been due to the fact that Aspergillus spores are more distributed in the atmosphere, their ability to grow in abundance everywhere and the production of small conidia that easily penetrate deep in the alveoli region and grow at 37°C. In addition, another study by (Xu et al. 2019) in China showed a high percentage of *Aspergillus* spp., among patients with chronic obstructive pulmonary disease (COPD) due to TB.

Prakash et al. (2019) in India reported that the incidence of mucormycosis is rising globally with the change in the epidemiology of mucormycosis in Asia, especially in India and China mostly among patients with uncontrolled diabetes mellitus.

Among sex of the patients with positive pathogenic fungal isolates, males were found to be 18 (60%) and females 12 (40%), as shown in Table 2.

Table 2
Distribution of pathogenic fungal among both sex

Fungal genus	Male		Female		Frequency/ 30	%
Candida spp.	14	70%	6	30%	20	66.7%
Aspergillus spp.	2	40 %	3	60%	5	16.7%
Cryptococcus neoformans	1	25%	3	75%	4	13.3 %
Mucor spp.	1	100%	0	0	1	3.3 %
Total	18	60 %	12	40 %	30	100%

There was no significant association between sex and the prevalence of fungal pathogens (P= 0.553), it agrees with (Sani et al., 2020), who confirmed that males had a relatively higher prevalence of fungal isolates,(63.5%) than females counterpart (36.5%) and (Wood et al., 2019) who stated that this fungal disease affects men (75.5%) often more than women. While it disagrees with (Njovu et al. 2021) in Uganda who reported that females 62 (59.3%) were more affected than males 46 (40.7%). Aluyi et al., (2010) and Ekenna et al., (2007) reported that the occurrence of pulmonary mycoses was higher in females 86(44.1%) than in males 80(41.0%), this discrepancy in prevalence can be attributed to geographical location, sample size, and diagnostic approaches used. Findings from the current study revealed that the higher colonization rates in males could have been responsible for increased risk among patients because they mostly engage in outdoor activities that may predispose them to inhalation of more fungal spores (Bulpa et al., 2017).

3.3. Relation between Culture and Direct Methods

By considering culture as a gold standard when compared with direct examination, the latter revealed high specificity as (88.5%) and low sensitivity as (60%) as shown in Table 3, which agree with a study by (Shamly, et al., 2014), who reported the specificity of the direct method was (93.0 %) and sensitivity (65.2%). While it disagrees with (Yadav et al., 2013) in India, who say that the specificity of the direct method was (40%) and (68%) sensitivity, the low sensitivity of direct method may come from the presence of collagen fibers, tissue matrix and air bubbles are often difficult to distinguish from fungi. The current study shows a high specificity (88.5%) and low sensitivity (60%), discrepancy of these methods might be due to the volume of (BAL), the volume of saline to retrieve cells from the pulmonary parenchyma, positioning of the patient and processing of the BAL fluid for cellular analyses.

Direct microscopic examination of candida in (BAL) fluid is more sensitive than culture, but utilization is limited as it requires expertise for interpretations and is distinguished poorly among fungi with similar morphology. Culture is still a gold standard compared to the direct method for isolated fungi.

Table 3
Sensitivity and specificity, positive and negative predictive value results of cultures as a gold standard compared to direct method

		Culture (gold standard)		Total
	Data analyzed	Positive	Negative	
Smear	Positive	18	10	28
	Negative	12	77	89
	Total	30	87	117
	Effect size	Value	95% CI	
	Sensitivity	60	42.32 - 75.41	
	Specificity	88.51	80.12 - 93.64	
	Positive Predictive Value	64.29	45.83 - 79.29	
	Negative Predictive Value	86.52	77.90 - 92.12	

3.4. Molecular Methods

Out of 117 BAL samples showed 27 (23%) for ITS were positive, as shown in Figure 4.

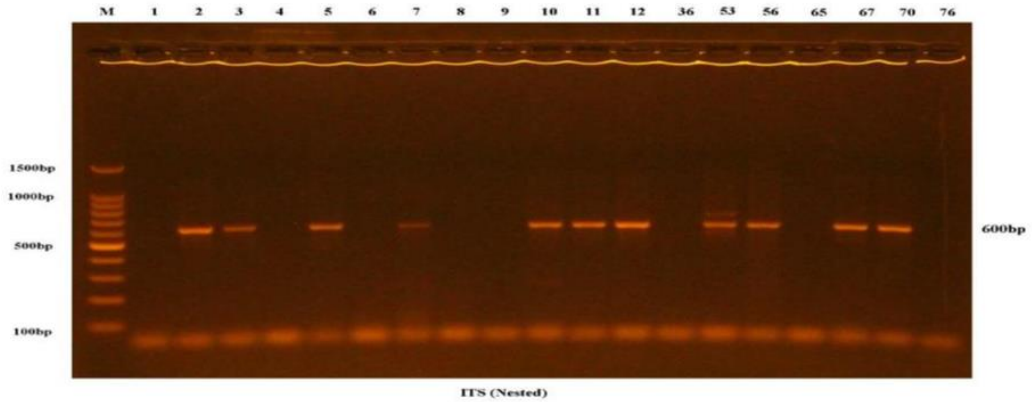

Fig. 4. Amplification of ITS gene of unknown fungal species were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-76 resemble ≈600bp PCR products

It nears to agree with a study done by (Badiee et al., 2019) in India in which 53/300 (17.7%) patients were positive for fungal PCR while not comparable to a study done by (Wagner *et al.* 2018) in Switzerland with 76/143 (53%)

positive ITS .These variations in the results could be attributed to the different sample sizes, demographics, differences in host factors, sample collection methods, and DNA extraction methods.

Fungal PCR for species-specific primer can detect fungal DNA even from nonviable cells with good sensitivity and specificity (Vilgalys et al., 1990). In the ongoing study, out of 27 (23%) ITS positive samples, 24 (20.5%) of there were PCR positive using fours specific primers for *Candida* spp., *Aspergillus* spp., *Cryptococcus* spp. *and Mucor* spp.. The results were seen that 14 (58.2%) samples belong to *Candida spp.,* 6(25.2%) samples belong to *Aspergillus* spp. and 2 (16.6%) samples for bath *Cryptococcus* spp. and *Mucor* spp. as distributed in Figure 5.

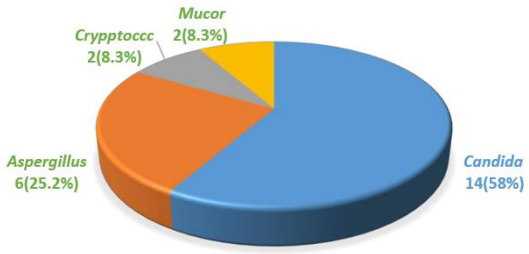

Fig. 5. Frequency of fungal among patients with respiratory disease detected by using housekeeping gene with conventional PCR

In this study, the result shows that the most commonly diagnosed fungi is *Candida* spp. followed by *Aspergillus* spp. This is in agreement with results done by (Spahr et al., 2019) also a study in Iran by (Zarrinfar et al., 2015), who reported that *Candida* spp. is the first most important cause of pulmonary fungal disease. Moreover, 6 patients (25%) belonged to *Aspergillus* spp.; distribution of Aspergillus in the environment can facilitate exposure and increase the risk of colonization and infection. Several recent reviews have highlighted the increasing incidence of Aspergillus infections that are associated with critical care medicine due to immunological (Zarrinfar et al., 2015).

Regarding detection method using housekeeping gene to detect the genus of fungus in BAL samples directly was as follows: in *Candida* spp. the *cavps13* gene amplification showed a product size 741 bp as represented in Figure 6, the housekeeping gene (*BGT1*) which is useful in the detection of *Aspergillus* spp., the product of this gene was 811 bp as in Figure 7, Regarding *Cryptococcus* spp., the *PLB1* gene was used and the PCR product appeared was about 532 bp as shown in Figure 8,while *Mucor* spp .the *MucL1* gene was used and its products were 836 bp which is explained in Figure 9.

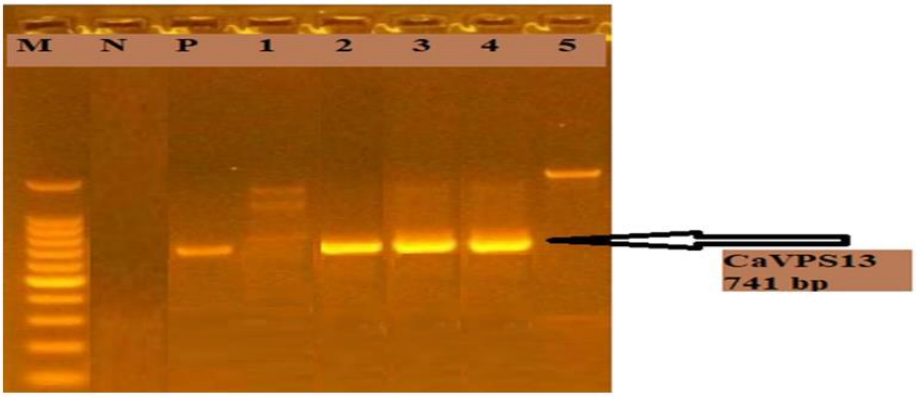

Fig. 6. Agarose gel electrophoresis of 741 bp specific PCR product for CaVP13 gene for candida spp. using 1.5% agarose gel at 90V for 1hr. in 1x TBE buffer, and visualized under transilluminator UV after staining by ethidium bromide. Lane M: 100 bp . DNA ladder, lane (2, 3, 4): positive result, lane N: negative control and lane P: positive control.

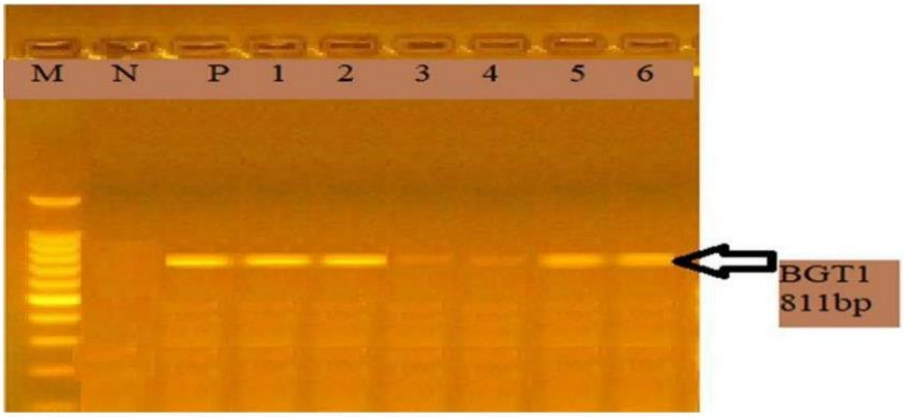

Fig. 7. Agarose gel electrophoresis of 811 bp specific PCR product for BGT1 gene for Aspergillus spp. using 1.5% agarose gel at 90V for 1hr. in 1x TBE buffer, and visualized under transilluminator UV after staining by ethidium bromide. Lane M: 100 bp. DNA ladder, lane (1, 2, 3, 4, 5, 6): positive result, lane N: negative control and lane P: positive control

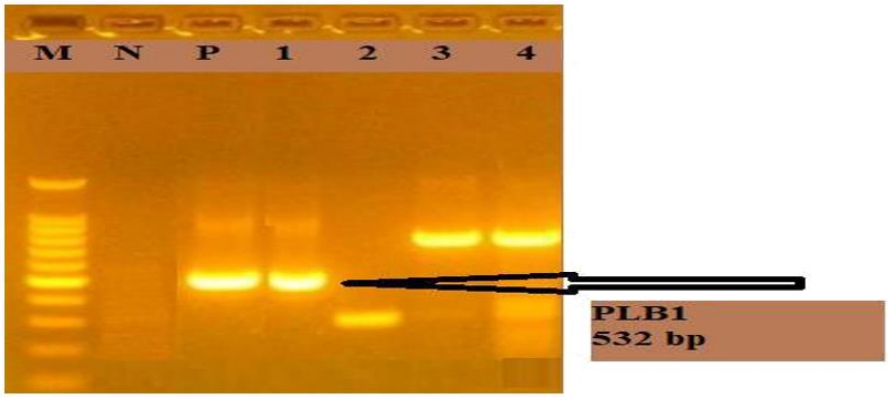

Fig. 8. Agarose gel electrophoresis of 532 bp specific PCR product for PLB1 gene for Cryptococcus spp.using 1.5% agarose gel at 90V for 1hr. in 1x TBE buffer, and visualized under transilluminator UV after staining by ethidium bromide. Lane M: 100 bp DNA ladder, lane (1): positive result, lane N: negative control and lane P: positive control

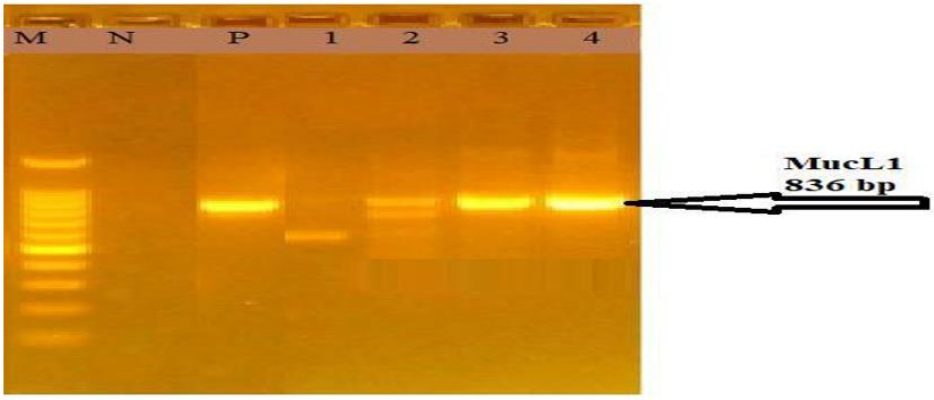

Fig. 9. Agarose gel electrophoresis of 836 bp specific PCR product for MucL1 gene for Mucor spp. using 1.5% agarose gel at 90V for 1hr. in 1x TBE buffer and visualized under transilluminator UV after staining by ethidium bromide. Lane M: 100 bp DNA ladder, lane (2, 3, 4): positive result, lane N: negative control and lane P: positive control

The results in PCR versus culture methods in this study showed that out of 117 samples fungal infection as 30(25.6 %) were detected by culture method, while the 27(23.0%) samples were detected by ITS technique as shown in Figure 10.

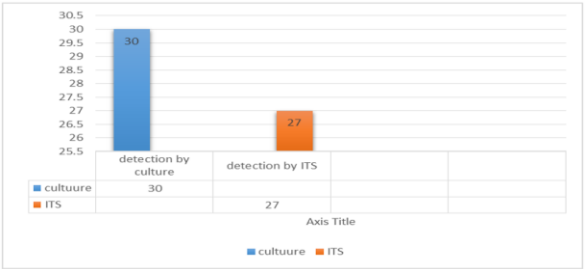

Fig. 10. Result of culture compared to conventional PCR(ITS) technique

3.5. Relation between Molecular Technique and culture Methods

By comparing PCR (ITS) with the culture method, the results of the current study revealed 26 samples were truly positive, while 86 samples were true negative, also 4 samples as false positive and only one sample was false negative as shown in Table 4.

Table 4
Positive and negative results of culture compared to ITS

		Culture		Total
		-Ve	+Ve	
ITS	-Ve	86(TN)	4 (FP)	90
	+Ve	1 (FN)	26 (TP)	27
Total		87	30	117

Sensitivity and specificity of ITS (PCR) compare to culture as a gold standard method, the results showed, the sensitivity was (86.6%), specificity (98.8%) of ITS detection technique with negative predictive value NPV (95.5%) while positive predictive value PPV was (96.3%) as shown in Table 5.

Table 5
Sensitivity and specificity of culture diagnostic method compared to ITS

Statistic	Value
Sensitivity	86.67
Specificity	98.8%
PPV	96.3%
NPV	95.5%

These results are in agreement with study conducted by (Capoor et al., 2017) who found that broad-range PCR showed a sensitivity, specificity, PPV positive predictive value and NPV negative predictive value was (94.3%) ,(95.2%), (97.6%) and (88.9%) respectively. Also another study by (Srinivas et al.2021), reported that the sensitivity, specificity, positive predictive value and negative predictive value of pan fungal PCR assay were found to be (82.7%) ,(90.48%), (92.31%) and (79.17%) respectively, a wide range of sensitivity seen in this study may be due to different case definitions and criteria for a PCR positive episode.

While it disagrees with study by (Lass-Flörl., et al., 2013) in Austria who found that broad-range PCR with a sensitivity, specificity, positive predictive value and negative predictive value was (57.1%), (97.0%), (80%) and (91.7%) respectively. The low sensitivity in this study may be due to species-specific PCR being based on the amplification of species-specific genes and usage is restricted to a limited number of pathogens from defined clinical samples.

The low sensitivity of the culture may be due to the contamination and slow growing of fungi, the patients who underwent BAL sampling may in the early stage of the disease, fungus from non-sterile specimens (BAL) may also reflect colonization of the airway instead of invasive infection. Culture is still considered the gold standard, but it is time-consuming, taking 1to3 days to grow and delaying the species identification, those that are slow to sporulate (Zarrinfar et al. 2015).

Culture remains the gold standard for the diagnosis of candida and has high sensitivity, while the Aspergillus is low, but the positive predictive value of culture depends on the prevalence of the infection; it is higher among immune-compromised patients and in areas of endemicity. As a general rule, isolation of *Aspergillus* spp. from BAL almost invariably represents colonization in immune-competent patients, while it suggests invasive disease in the immune-compromised patients (Rampini, et al., 2016).

The main advantage of PCR based detection is its diagnostic speed, prompt identification of species, the ability to allow rapidly for a species-targeted treatment, prospects for automation to decrease the contamination (Rampini, et al., 2016). Specific-species primer and the processed of cell wall disruption for DNA extraction which confirmed the diagnosis; also DNA may have been detected at an early stage of infection in some patients before clinical signs of disease.

4. Conclusion

The current study showed the percentage of respiratory fungal infection was 30 (25%) which remain a major cause of morbidity and mortality in certain high-risk groups. In this study, among patients with DM (diabetic mellitus) are more susceptible to develop fungal respiratory infection. Direct method for the detection of fungal infection among patients with respiratory disease was found to be more specificity (88%) and less sensitivity (60%) when compared with culture(gold standard method), referring to fungal species which was detected by PCR using specific-species primers include: *Candida* spp. *Aspergillus* spp., *Cryptococcus* spp. and *Mucor* spp.it was seen that, *Candida* spp. and *Apergillus* spp. had the highest positive percentages among other species and finally PCR (ITS) assay showed a high rate of specificity (98.8%) and sensitivity (86.6%) if compared to culture, when the latter considered to be the gold standard method for fungal infection.

Competing Interests

The authors have declared that no competing interests exist.

References

Ahmed, M. M., Farghaly, A. A., Raafat, R. H., & Abd Elsattar, W. M. (2019). Study of the prevalence and pattern of fungal pneumonias in respiratory intensive care units. Egyptian Journal of Bronchology, 13(4), 545-550. **https://doi.org/10.4103/ejb.ejb_37_19**

Akram, Z., Al-Kheraif, A. A., Kellesarian, S. V., Vohra, F., & Javed, F. (2018). Comparison of oral Candida carriage in waterpipe smokers, cigarette smokers, and non-smokers. Journal of Oral Science, 60(1), 115-120. **https://doi.org/10.2334/josnusd.17-0090**

Aluyi, H. S. A., Otajevwo, F. D., & Iweriebor, O. (2010). Incidence of pulmonary mycoses in patients with acquired immunodeficiency diseases. Nigerian Journal of Clinical Practice, 13(1). **https://www.ajol.info/index.php/njcp/article/view/53199**

Arvanitis, M., Anagnostou, T., Fuchs, B. B., Caliendo, A. M., & Mylonakis, E. (2014). Molecular and nonmolecular diagnostic methods for invasive fungal infections. Clinical Microbiology Reviews, 27(3), 490-526. **https://doi.org/10.1128/CMR.00091-13**

Baddley, J. W., Winthrop, K. L., Patkar, N. M., Delzell, E., Beukelman, T., Xie, F., ... & Curtis, J. R. (2011). Geographic distribution of endemic fungal infections among older persons, United States. Emerging infectious diseases, 17(9), 1664. **https://doi.org/10.3201%2Feid1709.101987**

Badiee, P., Kordbacheh, P., Alborzi, A., Malekhoseini, S., Ramzi, M., Mirhendi, H., ... & Shakiba, E. (2009). Study on invasive fungal infections in immunocompromised patients to present a suitable early diagnostic procedure. International Journal of Infectious Diseases, 13(1), 97-102. **https://doi.org/10.1016/j.ijid.2008.04.011**

Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., & Kauserud, H. (2010). ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC microbiology, 10(1), 1-9. **https://doi.org/10.1186/1471-2180-10-189**

Bezdicek, M., Lengerova, M., Ricna, D., Weinbergerova, B., Kocmanova, I., Volfova, P., ... & Racil, Z. (2016). Rapid detection of fungal pathogens in bronchoalveolar lavage

samples using panfungal PCR combined with high resolution melting analysis. Medical Mycology, 54(7), 714-724. https://doi.org/10.1093/mmy/myw032

Bulpa, P., Dive, A., & Sibille, Y. (2007). Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease. European Respiratory Journal, 30(4), 782-800. https://doi.org/10.1183/09031936.00062206

Cao, A. M. Y., Choy, J. P., Mohanakrishnan, L. N., Bain, R. F., & van Driel, M. L. (2013). Chest radiographs for acute lower respiratory tract infections. The Cochrane Database of Systematic Reviews, 2013(12). https://doi.org/10.1002/14651858.CD009119.pub2

Capoor, M. R., Puri, S., Raheja, H., Mohindra, R., Gupta, D. K., Verma, P. K., & Chowdhary, R. (2017). Screening of invasive fungal infections by a real-time panfungal (pan-ACF) polymerase chain reaction assay in patients with haematological malignancy. Indian Journal of Medical Microbiology, 35(1), 41-47. https://doi.org/10.4103/ijmm.IJMM_15_543

Chadana, M., Oberoi, L., & Malhotra, A. (2019). Emerging Fungal Respiratory Tract Infections in a Tertiary Care Hospital of North India, Punjab. Journal of Medical Science and Clinical Research, 7(8), 841-844. http://216.10.240.19/v7-i8/143%20jmscr.pdf

Ekenna, O., Uba, A., Chikwem, J. O., Mambula, S., Aliyu, M. B., & Mohammed, I. (2007). Relevance of moldy fungi as agents of chronic lower respiratory tract infection in patients seen in Maiduguri, Nigeria. West African journal of medicine, 26(2), 117-120. https://europepmc.org/article/med/17939312

Fajarningsih, N. D. (2016). Internal Transcribed Spacer (ITS) as DNA barcoding to identify fungal species: a review. Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology, 11(2), 37-44. https://doi.org/10.15578/squalen.v11i2.213

Jahromi, S. B., & Khaksar, A. A. (2002). FUNGAL ISOLATES OF THE RESPIRATORY TRACT: 460. Critical Care Medicine, 30(12), A111.

Jha, B. J., Dey, S., Tamang, M. D., Joshy, M. E., Shivananda, P. G., & Brahmadatan, K. N. (2006). Characterization of Candida species isolated from cases of lower respiratory tract infection. Kathmandu University medical journal (KUMJ), 4(3), 290-294. https://europepmc.org/article/med/18603921

José, R. J., Periselneris, J. N., & Brown, J. S. (2020). Opportunistic bacterial, viral and fungal infections of the lung. Medicine, 48(6), 366-372. https://doi.org/10.1016/j.mpmed.2020.03.006

Lao, M., Li, C., Li, J., Chen, D., Ding, M., & Gong, Y. (2020). Opportunistic invasive fungal disease in patients with type 2 diabetes mellitus from Southern China: clinical features and associated factors. Journal of Diabetes Investigation, 11(3), 731-744. https://doi.org/10.1111/jdi.13183

Lass-Flörl, C., Mutschlechner, W., Aigner, M., Grif, K., Marth, C., Girschikofsky, M., ... & Nachbaur, D. (2013). Utility of PCR in diagnosis of invasive fungal infections: real-life data from a multicenter study. Journal of clinical microbiology, 51(3), 863-868. https://doi.org/10.1128/JCM.02965-12

Lengerova, M., Racil, Z., Hrncirova, K., Kocmanova, I., Volfova, P., Ricna, D., ... & Mayer, J. (2014). Rapid detection and identification of mucormycetes in bronchoalveolar lavage samples from immunocompromised patients with pulmonary infiltrates by use of high-resolution melt analysis. Journal of Clinical Microbiology, 52(8), 2824-2828. https://doi.org/10.1128/JCM.00637-14

Louisiana Office of Public Health – Infectious Disease Epidemiology Section – Annual Report. Cryptococcosis. 2010.

Mandell, G. L., Douglas Jr, R. G., & Bennett, J. E. (1979). Principles and practice of infectious diseases. Volumes 1 and 2. John Wiley & Sons.

Mikulska, M., Furfaro, E., De Carolis, E., Drago, E., Pulzato, I., Borghesi, M. L., ... & Viscoli, C. (2019). Use of Aspergillus fumigatus real-time PCR in bronchoalveolar lavage samples (BAL) for diagnosis of invasive aspergillosis, including azole-resistant cases, in high risk haematology patients: the need for a combined use with galactomannan. Medical mycology, 57(8), 987-996. https://doi.org/10.1093/mmy/myz002

Njovu, I. K., Musinguzi, B., Mwesigye, J., Kassaza, K., Turigurwa, J., Nuwagira, E., ... & Itabangi, H. (2021). Status of pulmonary fungal pathogens among individuals with clinical features of pulmonary tuberculosis at Mbarara University Teaching Hospital in Southwestern Uganda. Therapeutic Advances in Infectious Disease, 8, 20499361211042477. https://doi.org/10.1177/20499361211042477

Njovu, I. K., Musinguzi, B., Mwesigye, J., Kassaza, K., Turigurwa, J., Nuwagira, E., ... & Itabangi, H. (2021). Status of pulmonary fungal pathogens among individuals with clinical features of pulmonary tuberculosis at Mbarara University Teaching Hospital in Southwestern Uganda. Therapeutic Advances in Infectious Disease, 8, 20499361211042477. https://doi.org/10.1177/20499361211042477

Ogba, O. M., Abia-Bassey, L. N., & Epoke, J. (2013). The relationship between opportunistic pulmonary fungal infections and CD4 count levels among HIV-seropositive patients in Calabar, Nigeria. Transactions of the Royal Society of Tropical Medicine and Hygiene, 107(3), 170-175. https://doi.org/10.1093/trstmh/trs025

Pendleton, K. M., Huffnagle, G. B., & Dickson, R. P. (2017). The significance of Candida in the human respiratory tract: our evolving understanding. Pathogens and Disease, 75(3), ftx029. https://doi.org/10.1093/femspd/ftx029

Prakash, H., & Chakrabarti, A. (2019). Global epidemiology of mucormycosis. Journal of Fungi, 5(1), 26. https://doi.org/10.3390/jof5010026

Puebla, L. E. J. (2012). Fungal infections in immunosuppressed patients. Immunodeficiency.

Rafat, Z., Hashemi, S. J., Ashrafi, K., Nikokar, I., Jafari, A.,

Foroushani, A. R., ... & Najar-Shahri, N. (2020). Fungal isolates of the respiratory tract in symptomatic patients hospitalized in pulmonary units: a mycological and molecular epidemiologic study. Journal of Multidisciplinary Healthcare, 13, 661. **https://doi.org/10.2147%2FJMDH.S252371**

Rampini, S. K., Zbinden, A., Speck, R. F., & Bloemberg, G. V. (2016). Similar efficacy of broad-range ITS PCR and conventional fungal culture for diagnosing fungal infections in non-immunocompromised patients. BMC microbiology, 16(1), 1-8. **https://doi.org/10.1186/s12866-016-0752-1**

Rodrigues, C. F., Rodrigues, M. E., & Henriques, M. (2019). Candida sp. infections in patients with diabetes mellitus. Journal of Clinical Medicine, 8(1), 76. **https://doi.org/10.3390/jcm8010076**

Roohani, A. H., Fatima, N., Shameem, M., Khan, H. M., Khan, P. A., & Akhtar, A. (2018). Comparing the profile of respiratory fungal pathogens amongst immunocompetent and immunocompromised hosts, their susceptibility pattern and correlation of various opportunistic respiratory fungal infections and their progression in relation to the CD4+ T-cell counts. Indian journal of medical microbiology, 36(3), 408-415. **https://doi.org/10.4103/ijmm.IJMM_18_258**

Sani, F. M., Uba, A., Tahir, F., Abdullahi, I. N., Adekola, H. A., Mustapha, J., ... & Daneji, I. M. (2020). Spectrum of pulmonary fungal pathogens, associated risk factors, and anti-fungal susceptibility pattern among persons with presumptive tuberculosis at Gombe, Nigeria. International Journal of Mycobacteriology, 9(2), 144. **https://doi.org/10.4103/ijmy.ijmy_46_20**

Saud, B., Bajgain, P., Paudel, G., Shrestha, V., Bajracharya, D., Adhikari, S., ... & Awasthi, M. S. (2020). Fungal infection among diabetic and nondiabetic individuals in Nepal. Interdisciplinary Perspectives on Infectious Diseases, 2020. **https://doi.org/10.1155/2020/7949868**

Shamly, V., Kali, A., Srirangaraj, S., & Umadevi, S. (2014). Comparison of microscopic morphology of fungi using lactophenol cotton blue (LPCB), iodine glycerol and congo red formaldehyde staining. Journal of clinical and diagnostic research: JCDR, 8(7), DL01. **https://doi.org/10.7860%2FJCDR%2F2014%2F8521.4535**

Spahr, J., Weiner, D. J., Stokes, D. C., & Kurland, G. (2019). Pulmonary disease in the pediatric patient with acquired immunodeficiency states. In Kendig's Disorders of the Respiratory Tract in Children (pp. 923-943). Elsevier. **https://doi.org/10.1016/B978-0-323-44887-1.00064-X**

Srinivas, S., Kumari, P., & Gupta, D. K. (2021). Utility of Panfungal PCR in the diagnosis of invasive fungal infections in febrile neutropenia. Journal of Family Medicine and Primary Care, 10(7), 2533. **https://doi.org/10.4103%2Fjfmpc.jfmpc_2325_20**

Vilgalys, R., & Gonzalez, D. (1990). Organization of ribosomal DNA in the basidiomycete Thanatephorus praticola. Current Genetics, 18(3), 277-280. **https://doi.org/10.1007/BF00318394**

Wagner, K., Springer, B., Pires, V. P., & Keller, P. M. (2018). Molecular detection of fungal pathogens in clinical specimens by 18S rDNA high-throughput screening in comparison to ITS PCR and culture. Scientific reports, 8(1), 1-7. **https://doi.org/10.1038/s41598-018-25129-w**

Wood, R. E. (2019). Bronchoscopy and bronchoalveolar lavage in pediatric patients. Kendig's Disorders of the Respiratory Tract in Children, 134-146. **https://doi.org/10.1016/B978-0-323-44887-1.00009-2**

Xu, L., Chen, B., Wang, F., Wei, C., Liu, H., Liu, J., ... & Luo, F. (2019). A higher rate of pulmonary fungal infection in chronic obstructive pulmonary disease patients with influenza in a large tertiary hospital. Respiration, 98(5), 391-400. **https://doi.org/10.1159/000501410**

Yadav, S., Saxena, A., Capoor, M., & Ramesh, V. (2013). Comparison of direct microscopic methods using potassium hydroxide, periodic acid Schiff, and calcofluor white with culture in the diagnosis of onychomycosis. Indian Journal of Dermatology, Venereology, and Leprology, 79(2), 242-242.

Zarrinfar, H., Mirhendi, H., Fata, A., Khodadadi, H., & Kordbacheh, P. (2015). Detection of Aspergillus flavus and A. fumigatus in bronchoalveolar lavage specimens of hematopoietic stem cell transplants and hematological malignancies patients by real-time polymerase chain reaction, nested PCR and mycological assays. Jundishapur Journal of Microbiology, 8(1). **https://doi.org/10.5812%2Fjjm.13744**