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## Survey Study of the Allergic Fungi in Kirkuk Area and Use Molecular Detection for Identification

Sanaa Huseein Mohamad<sup>a</sup>, Karkaz M. Thalij<sup>b</sup>, Khalil AL-Bander<sup>c</sup>, Batol Imran  
Dheeb<sup>d\*</sup>

<sup>a</sup>Kirkuk University, College of Science, Biology Department – Kirkuk/ Iraq

<sup>b</sup>Tikreet University, College of Agriculture, Food Science Department., <sup>3</sup>Tikreet University – Tikreet/ Iraq

<sup>c</sup>Tikreet University, College of Science, Biology Department – Tikreet/ Iraq

<sup>d</sup>Iraqia University, Biology Department- Baghdad/ Iraq

### Abstract

The study was carried out at the period from 1<sup>st</sup> February 2013 until 31<sup>st</sup> January 2014 in order to determine the relationship of the allergic fungi with the lower bronchial allergy patients and study the occurrence of fungal in indoor and outdoor building. Two hundred samples of sputum were collected from the respiratory tract infected patients and 20 samples sputum from healthy persons, which ranged in age from less than five to more than 51 years of both sexes the results showed that the rate of infection among examined cases were 134 (67%), by direct microscopy and culturing. The rate of infection in females was higher 78 (58.2%) than males 56 (41.7%). The distribution of positive cases were 51 (38.05%) due to the *Candida albicans* ; 46 (34.3%) *Aspergillus fumigatus* ; 18 (13.4%) *Aspergillus flavus* and 19 (14.1%) *Aspergillus niger*. Out of 200 swabs samples from polluted buildings; 120 (60%), were positive, most of positive samples were from outdoor (schools) 57 (47.5%) while 48(40%) in indoor (house) and 8(6.6%); 7(5.8%) in Hall of Science College and Student Campus (rooms) respectively.

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\* Corresponding author.

E-mail address: batoolomran@yahoo.com.

The diagnosis of some fungal organisms using specialized genes for both *A.fumigatus* and *C. albicans* in PCR technique, as assurance of the diagnosed with traditional methods were the frequency of the gene *Afumi* in all isolates of the *A.fumigatus* isolated with the molecular weight at 400 bp and the frequency of the gene *CABF59* was 100% on *C. albicans* yeast samples and molecular weight was at 700 bp.

**Keywords:** Allergic fungi; Outdoor; Indoor fungi; PCR

## 1. Introduction

Air borne fungi have been found to be associated with certain respiratory illnesses and allergies [1], while exposure to fungi and other microbes, their fragments and metabolites may constitute a health risk bronchial hyperactivity and other respiratory symptoms [2]. It has been estimated that more than 80 genera of fungi are linked with symptoms of respiratory tract allergies [3], with the most common allergenic genera being *Aspergillus*, *Candida*, *Fusarium*, *Cladosporium* and *Alternaria*.. Exposure to large concentrations of the spores of these genera causes aspergillosis [4], pneumonitis allergic alveolus and toxicosis [5]. *Aspergillus fumigatus* has become one of the most dangerous fungal pathogens for immunocompromised individuals with the risk of acquiring disease termed aspergillosis that is usually localized in the respiratory tract [6].

As a common sense, the main site of entry micro-organisms is the host's lung [7, 8, 9, 10, 11,12]. The primary infection results from inhalation of the air borne spores are minute and easily reach the alveoli of the respiratory system. Most patients suffering from aspergillosis have an impaired immune system that is often evoked by leukemia, neutropenia or after prolonged treatment with steroids such as solid organ transplantation patients. The mortality rate of aspergillosis among these patients lies between 30 to 90% [13, 14]. To a limited extent, immune-competent persons can acquire aspergillosis, but the number of incidence, is by far, not as high as compared to those immune-compromised patients [13]. The study aimed to isolation of allergic fungi from different sources and molecular identification.

## 2. Methods

### 2.1 Samples collection

sputum samples were collected from 200 patients (76 male, 124 female) ages ranged between (<5 >51 years old) suspected in being infected with asthma and aspergillosis (according to clinically identification by a physician), during the period of 1<sup>st</sup> February 2013 to 31<sup>st</sup> January 2014 from chest and "Respiratory Disease Specialized Center, Ministry of Health, Kirkuk Governorate (this specialized center generally accepted patients from different area of Kirkuk (Al-Dibis, Al- Riyad, Al-Hawija, Al-Altun -Kupri, and Taza-Khurmatu) as well as from nearby provinces of Salahaddin, Sulaymaniah and Mosul while floor dust samplings were performed by special wipes swabs with 6% of optimal medium in fixed diameters of polyester, which collected 200 sampling swabs from different buildings, indoor-patients houses43 (73) and outdoor from primary and secondary schools20 (105); Halls of Science of College8 (8) and Camps of Students (rooms) 10 (14), then cultured on SDA and malt yeast extract media at 28° C for 10 days.

The samples were examined directly under the microscope using 10% KOH solution and culturing on the SDA agar and examined for after 7 days [15, 16, 17, 18]. The isolates were identified according to [19, 20, 21].

## 2.2 Extraction of DNA from *A. fumigatus* and *C.albicans* isolates

The DNA was extracted from 0.5 gm. (dry weight) was transferred to a mortar, frozen in liquid nitrogen (-190) to freeze the fungus the powder was transferred into a 1.5 mL size eppendorf tube and mixed with 600 µL of extraction buffer at 50°C. The eppendorf tubes incubated at 50-60°C water bath for 60 minutes; mixed several times while it was incubated.

The samples were centrifuged at (4000 rpm) for 15 min, the upper phase was transferred into fresh eppendorf tube in the fume hood. 600µL of chloroform/ isoamyl alcohol (24:1) was added to each sample and repeated twice. The samples were shaken 5-10 minutes at room temperature (in the fume hood). Then the samples were centrifuged at (4000 rpm) for 15 minute, the upper phase was transferred by Micropipette into a fresh eppendorf tube and 600µL of chloroform was added to each sample this step repeated twice times. The samples were centrifuged at (4000 rpm) for 15 minute. The supernatant (Buffer contain DNA) was collected in eppendorf tube. A 600µL of cold isopropanol (-20°C) was added and covered tightly. the samples were then centrifuged at (12000 rpm) for 15 minute to get the DNA precipitate on the wall of eppendorf 50 µL of TE buffer was added to wash off the wall as DNA precipitate, concentration estimated of the DNA using nanodrop.

## 2.3 Technique

In PCR technique to detect gene responsible for pathogenic *A. fumigatus* and *C. albicans* a primer specific to *C. albicans* DNA and *A. fumigatus* genes was used. *Afumi* gene subjected to confirm the pathogenicity of *A. fumigatus* isolated from sputum of patients, *CABF59* gene subjected to confirm the pathogenicity of *C. albicans* isolated from sputum of patients.

- PCR reactions were performed using the molecular weight markers :

100 DNA ladder (Promega Corporation).

PCR primer for *Afumi*, *CABF59*.

The sequence of the forward and reverse primers of the *Afumi* gene and *C. albicans* were designed based on the published sequence strand for *A. fumigatus* and *C. albicans* [22, 23].

The PCR program was adopted following optimization to work at optimum condition towards achievement the best detection. No. of cycles = 30 cycles between initial denaturation and final extension, table 3 shows PCR program.

Approximately 12µL of PCR amplified products were separated by Electrophoresis in 1.2% agarose gels (2 hrs, 5V/cm, 1X Tris-borate buffer). Gels were stained with ethidium bromide; PCR products were visualized by UV illuminator and then were imaged by gel documentation system. The amplified products PCR products were then estimated by comparing with the marker DNA ladder (100-1500) bp.

**Agarose Gel Electrophoresis** to separate the DNA fragments, 1% of agarose gel in different concentrations was used to extract DNA and 1.2% for visual checking of specific PCR product electrophoresis buffer was added to cover for 1-2 hours at 5V/cm. Agarose gel was stained with ethidium bromide 0.5mg/ml for 20-30 minutes. DNA bands were visualized by UV trans illuminator at (302-320) nm wavelength. A gel documentation system was used to document the observed bands [24].

**Table 1:** Oligonucleotide primers used to amplify marker genes in *C. albicans* and *A. fumigatus* by PCR.

| Primer and target species | Name     | Direction (F/R) | Sequence                        |
|---------------------------|----------|-----------------|---------------------------------|
| <i>C. albicans</i>        | CABF59   | F               | 5-TTGAACATCTCCAGTTTCAAAGGT-3    |
|                           | CABR110  | R               | 5-GTTGGCGTTGGCAATAGCTCTG-3      |
|                           | CADBR125 | R               | 5-AGCTAAATTCATAGCAGAAAGC-3      |
| <i>A. fumigatus</i> ,     | Afumi    | F1              | 5- GCCCGCCGTTTCGAC-3            |
|                           | Afumi    | R1              | 5-CCGTTGTTGAAAGTTTTAACTGATTAC-3 |

**Table 2:** Volumes and concentrations of PCR mixture used in present study.

| Material            | Final Concentration | Volume for 1 tube |
|---------------------|---------------------|-------------------|
| PCR PreMix          | 1X                  | 5µl               |
| Deionized D.W       | —                   | 11µl              |
| Primer (10 pmol/µl) | 10 pmol/µl          | 2µl               |
| DNA template        | 100 ng              | 2µl               |

**Table 3:**PCR program used in the study.

| Steps                | Temperature | Time            |
|----------------------|-------------|-----------------|
| Initial denaturation | 94°C        | 10min           |
| Denaturation         | 94°C        | 0.3 min(30sec)  |
| Annealing            | 50°C        | 0.45 min(45sec) |
| Extension            | 72°C        | 1.15 min        |
| Final Extension      | 72°C        | 10 min          |

### 3. Results and discussion

#### 3.1 Isolation of fungi from the lower respiratory tract (LRT) of infected patients:

The present study involves isolation of infectious fungi collected from sputum specimens of patients with lower respiratory tract (LRT) infection. Only 200 samples were directly examined using 10% KOH solution from which a total of 134 samples were found positive while other, 66 samples were negative (table 4). Hyphae and dichotomous were detected as branched, conidial heads and chains basipetally from phialides, while chains of conidia and vesicles were born directly in the absence of metulae. Using the above Potassium hydroxide alkaline solutions (NaOH) the fungus samples would remain unaffected and easily recognizable from other mixed substances [16, 25]. Both, direct examination and culturing methods had produced same result. This indicates that both implemented procedures are accurate. The 66 negative samples were further considered as they might have been infected with other micro-organisms.

**Table 4:** Distribution of positive and negative cultured cases according to both diagnosing procedure adopted.

| Procedures                 | Samples | Positive samples |          |
|----------------------------|---------|------------------|----------|
|                            |         | No.              | %        |
| Direct examined by 10% KOH | 200     | 134              | 67a±0.00 |
| Culturing Procedure        | 200     | 134              | 67a±0.00 |

#### 3.2 Isolation of fungi from LRT infected patients according to fungal types:

The infection with *C.albicans* made up 51(38.05%) which represented the highest record detected; followed by *Aspergillus fumigatus* 46(34.3%). The latter made almost same frequency of *C.albicans*, while both *Aspergillus niger* and *Aspergillus flavus* had made up one fifth of infectious incidents as show in table (5).

**Table 5:** Distribution of positive cases according to fungal types.

| Fungal Species          | Number of Isolates |            |
|-------------------------|--------------------|------------|
|                         | No.                | %          |
| <i>Candida albicans</i> | 51                 | 38.05a±0   |
| <i>A. fumigatus</i>     | 46                 | 34.3b±0.3  |
| <i>A.flavus</i>         | 18                 | 13.4c±0.03 |
| <i>A.niger</i>          | 19                 | 14.1c±0.2  |
| Total                   | 134                | 100        |

The incidents of aspergillosis were proportionally increased in patients with impaired immune state associated with the management of malignancy, organ transplantation, autoimmune and inflammatory conditions; critically ill patients and those with chronic obstructive pulmonary disease (COPD). The latter may alert an increased risk

of *A. fumigatus* which could lead to variety of infectious diseases. The aspergillosis, depends on the host's immune statues primarily in patients with severe immunodeficiency [26]. Both *A. fumigatus* and *C.albicans* isolated from LRT patients with cystic fibrosis showed that *C.albicans* was higher than *A. fumigatus* [27]. However, others reported that in certain cases *A. fumigatus* can contribute to the pathogenicity of the fungi [28] while it was considered the main causative agent of aspergillosis in immunocompromised individuals [29, 30, 31]. The current result is in accordance with [27] regarding the highest infection with *C. albicans* while it is concomitant with [28, 29, 30, 31] as a causative agent of aspergillosis.

**3.3 Distribution of molds and yeast in infected patients with LRT according to Gender:**

There has been significant difference ( $p \leq 0.001$ ) of higher distribution of molds and yeast generally in female patients 78(58.2%) than in male 56(41.7 %) [Table 4-3].The highest infection was shown among *C.albican* sin female 32(62.7%) than in male 19(37.2%); 24(52.1%) in female than in male 22(47.8%) for *A.fumigatus*, 11(61.1%),7(38.8%) to *A.flavus* and 11(57.8%) ,8(42.1%) to *A. niger*, respectively. The latter represented almost 1.5 folds in female than male. Despite the difference in methodology being adopted researches involve these fungi in human are so scanty. The reason of such higher occurrence of LRT infection in females in comparison with males is concomitant with that of [32] who found that aspergillosis in female was almost twice than in male patients with ratio range 20:10 between male and female. In the present study the higher infection of female may be related to defect of health care and to the genetic factor of female which leads to decrease the immunity at old age. Although the present work showed the frequency of aspergillosis, generally, in female is higher than in male which covered 4 genera; however, [31] worked only on *A.fumigatus* found the infection frequency was opposite way around.

**Table 6:** Distribution of positive samples, fungal isolates according to the gender.

| Sex    | <i>C. albicans</i> |           | <i>A. fumigatus</i> |            | <i>A. flavus</i> |             | <i>A. niger</i> |            | Total |       |
|--------|--------------------|-----------|---------------------|------------|------------------|-------------|-----------------|------------|-------|-------|
|        | No.                | %         | No.                 | %          | No.              | %           | No.             | %          | No.   | %     |
| Male   | 19                 | 37.2b±0.3 | 22                  | 47.8b± 0.2 | 7                | 38.8b± 0.05 | 8               | 42.1b± 0.1 | 56    | 41.7b |
| Female | 32                 | 62.7a±0.6 | 24                  | 52.1a± 0.1 | 11               | 61.1a± 0    | 11              | 57.8a± 0.1 | 78    | 58.2a |
| Total  | 51                 | 38.05     | 46                  | 34.3       | 18               | 13.4        | 19              | 14.1       | 134   | 100   |

**3.4 Distribution of both molds and yeast detected from LRT patients according to their age**

The distribution of fungi showed higher infection of *Candida albicans* in age group ranged >50years while the lower infections were at ages lower than 5 years old. In general, the higher infection was starting from 25 years old of age and above (Table4).

Lower respiratory infection was found to be more prevalent in >51 years old [27, 31, 32, 33] . These results are in concomitant with the present study. The prevalence of the disease was proportional with certain cases i.e.

impaired immune state associated with the management of malignancy, organ transplantation, autoimmune and inflammatory conditions; critically ill patients and those with chronic obstructive pulmonary disease [COPD] [26].

**Table 7:** Distribution of both molds and yeast detected from the infected LRT of patients according to age

| Age patients (Years) | Fungal species     |            |                     |            |                  |           |                 |           | Total | %    |
|----------------------|--------------------|------------|---------------------|------------|------------------|-----------|-----------------|-----------|-------|------|
|                      | <i>C. albicans</i> |            | <i>A. fumigatus</i> |            | <i>A. flavus</i> |           | <i>A. niger</i> |           |       |      |
|                      | No.                | %          | No.                 | %          | No.              | %         | No.             | %         |       |      |
| 4 -5                 | 0                  | 7.8e± 0    | 0                   | 0± 0       | 0                | 0± 0      | 0               | 0± 0      | 4     | 2.9  |
| 6 - 12               | 7                  | 13.7c±0.08 | 0                   | 0±0        | 0                | 0± 0      | 0               | 0±0       | 7     | 5.2  |
| 13 - 24              | 5                  | 9.8d±1.1   | 2                   | 4.3c±1.1   | 0                | 0± 0      | 1               | 5.2c± 1.1 | 8     | 5.9  |
| 25 - 50              | 17                 | 33.3b±1.1  | 15                  | 32.6b± 0.5 | 4                | 22.2b±1.1 | 5               | 26.3b±0.5 | 41    | 30.5 |
| 51 >                 | 18                 | 35.2a±1.1  | 29                  | 63.0b±2.5  | 14               | 77.7a±1.1 | 13              | 68.4a±1.7 | 74    | 55.2 |
| Total                | 51                 | 38.05      | 46                  | 34.3       | 18               | 13.4      | 19              | 14.1      | 134   | 100  |

**3.5 The correlation between the incidences of (LRT) infection with type of patient's accommodation:**

Statistical analysis built on questionnaire illustrated that those patient lived in different house type Stone, Brick, Block; Clay had significant different degree of infection amongst them. Those who lived in block had the highest percentage 106(70.6%) followed by Stone 14(58.3%), Clay8 (57.1%) and Brick6 (50%) respectively (table 4-5).

The contaminations detected in these dwellings could be attributed to a number of different factors, including poor hygiene, the accumulation of dust, insufficient or intermittent air conditioning, poor ventilation and the presence of substrates providing suitable nutrients for fungal development e.g. wood, cellulose and textiles which would increase in microbial concentrations [34, 35, 36]. This was the case in this study as either one or all of those reasons had contributed accumulatively to the results.

**3.6 The comparison between indoor and outdoor according to fungal types:**

Two hundred swabs were collected from five building in Kirkuk, included outdoor (Primary School, Secondary School, Hall of Science College, Student Campus (rooms),and indoor (Total house) during the period (1<sup>st</sup>Februaryuntil 31Janaruary).Microscopic examination and culturing illustrates that 120,of them were

contaminated with fungi as show in table(4-6).Where is cultural identification showed that the higher contamination in outdoor 57 (47.5%) ; 8(6.6%);7(5.8%) than indoor48(57%) ,as well as recorded the highest percentage in *A.niger*26 (21.6%) followed by *Penicillium spp* 21(17.5%); *A. Flavus*19 (15.8%); *Alternaria spp* 17(14.1%); *Microsporium*spp and *Fusarium spp*11(9.1%); *A. fumigatus* 9(7.5%) and *Trichophyton spp.* (Dermatophytic fungi 6(5%))respectively the figure (4-6) shows the image which the fungi isolates during this study . [37] who showed the building frame material had more importance on fungal concentrations than moisture damage, the concentrations were higher in building of Wooden construction than in those with concrete / brick construction, the moisture damage significantly increased the concentrations of fungi in buildings of concrete /brick construction. The most common fungal in school buildings were *Penicillium* ,*Yeasts*, *Cladosporium* and *Aspergillus* ,we also showed the moisture damage was a risk factor for children's respiratory symptoms in both school types combined and in concrete / brick schools separately, also higher fungal concentrations in Wooden buildings has also reported by [38], although he did not separate the importance of moisture damage on the concentrations of fungi. In general, fungal concentrations detected in indoor air of schools were low compared with those previously found in Finnish homes [39, 40] , but correspond to the levels reported from schools in Norway [41].

**Table 8:** Sampling according to type of patient's house

| Type of House building | Total Samples | No. of positive samples | %          |
|------------------------|---------------|-------------------------|------------|
| Stone                  | 24            | 14                      | 58.3b± 1.1 |
| Brick                  | 12            | 6                       | 50c± 0.5   |
| Block                  | 150           | 106                     | 70.6a± 1.7 |
| Clay                   | 14            | 8                       | 57.1b± 1.9 |
| Total                  | 200           | 134                     | 67         |

In this study, the contaminations detected of these buildings were very old, with highly moisture, overcrowded and in a poor condition, which all encourage fungi to grow quickly. In addition, existence of some nearby plants i.e. Kirkuk Cement Factory, North Gas Company and North Oil Company have added bonus factors to over contaminate the surrounding air. Consequently, the inhaled air would ease infection of the respiratory system and may affect the body immunity. The potentially pathogenic *A.flavus* ,*Penicillium spp* ,*Fusarium spp* ,*Candida albicans* and *Alternaria spp* have already been isolated from indoor air a teaching hospital wards in Nigeria by [42]. Hospital associated infections were linked with many factors among which is the microbial quality of the indoor air of different wards and units of each hospital [43, 44], which reported that phthalate exposure along with some commonly present bio-contaminants in general house environments could both be important risk factors on the incidence of children's respiratory and allergic symptoms [45]. They found indoor mycobiota in a range of public and private buildings (libraries, museums, laboratories and offices warehouses, homes and a school) in the city of Havana(Cuba). At least 28 genera and 31 species identified included



*Aspergillus flavus*, *Aspergillus niger*, *Penicillium citrinum*, *Cladosporium cladosporioides* and *Cladosporium sphaerospermum*. Similar findings were reported by other authors in Cuba [45, 46, 47, 48]. They correlated these cosmopolitan genera to geography of Havana while attributed the variations in abundance to other materials contaminated at sampling points; in addition to the prevailing conditions at the time of sampling. Similar findings were seen elsewhere [36, 49, 50]. The predominance of *Aspergillus* at 80% of sampling points dose indicate that relative humidity levels of over 68% could favor the development of this fungus, which is reported to be a primary colonizer readily releasing spores at an air velocity as low 0.5 ms<sup>-1</sup>[51].

**Table 9:** The comparison between indoor and outdoor according to fungal types

| Fungal Species<br>Diagnosis | Indoor<br>fungi<br>(house) |               | Outdoor fungi |               |                               |           |                            |           |       |      |
|-----------------------------|----------------------------|---------------|---------------|---------------|-------------------------------|-----------|----------------------------|-----------|-------|------|
|                             |                            | %             | Schools       | %             | Hall of<br>science<br>college | %         | Student<br>Campus<br>rooms | %         | Total | %    |
| <i>A.fumigatus</i>          | 8                          | 16.6c±0.5     | 0             | 0e±0          | 0                             | 0c±0      | 1                          | 14.2b±2.1 | 9     | 7.5  |
| <i>A.niger</i>              | 12                         | 25a±0         | 10            | 17.5b±1.0     | 2                             | 25a±0     | 2                          | 28.5a±3.0 | 26    | 21.6 |
| <i>A.flavus</i>             | 9                          | 18.7b±<br>0   | 10            | 17.5b±0.7     | 0                             | 0c±0      | 0                          | 0c±0      | 19    | 15.8 |
| <i>Penicillium</i> spp      | 8                          | 16.6c±<br>1.1 | 11            | 19.2a±0       | 1                             | 12.5b±1.5 | 1                          | 14.2b±7.1 | 21    | 17.5 |
| <i>Alternaria</i> spp       | 2                          | 4.1f±0.5      | 12            | 21.0a±<br>0.5 | 2                             | 25a±1.5   | 1                          | 14.2b±2.8 | 17    | 14.1 |
| <i>Trichophyton</i><br>spp. | 2                          | 4.1f± 1.7     | 4             | 7.01d±1.1     | 0                             | 0c±0      | 0                          | 0c±0      | 6     | 5    |
| <i>Microsporium</i><br>spp. | 3                          | 6.2e± 1.2     | 6             | 10.5c±0       | 1                             | 12.5b±1.5 | 1                          | 14.2b±2.1 | 11    | 9.1  |
| <i>Fusarium</i> spp.        | 4                          | 8.3d± 0       | 4             | 7.01d±0.5     | 2                             | 25a±1.5   | 1                          | 14.2b±2.1 | 11    | 9.1  |
| Total                       | 48                         | 40            | 57            | 47.5          | 8                             | 6.6       | 7                          | 5.8       | 120   | 100  |

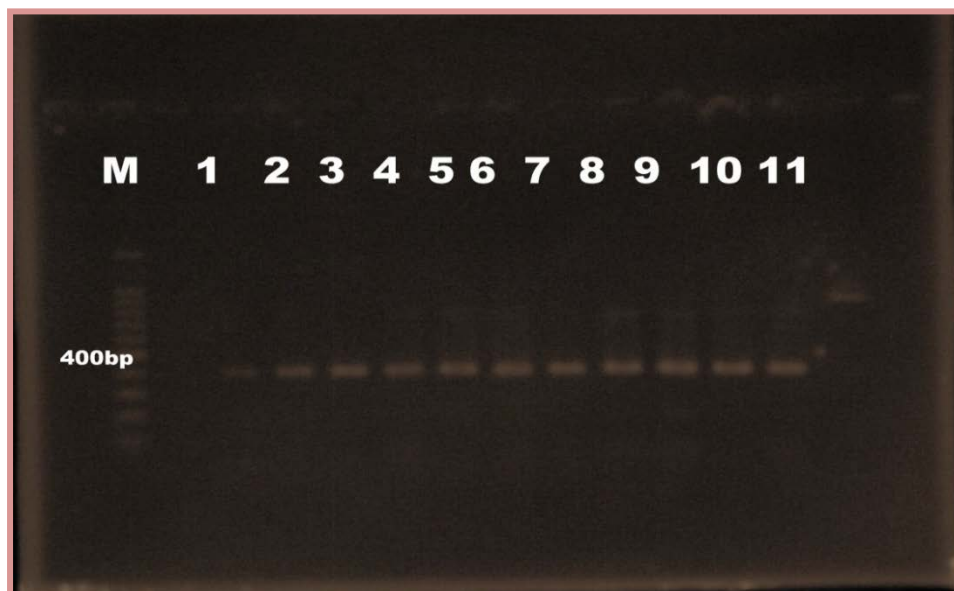
In terms of the biosafety levels suggested by [52], *A.flavus* and *A.fumigatus* would be considered the species of most harmful to human health; however, *A.niger*, *A.terreus*, *A.tamaritii* and *C.cladosporioides* have also been implicated in a range of pathologies [53]. Appropriate measures should therefore be implemented to reduce fungal density in the environments tested with a view to improving air quality and avoiding potential adverse effects.

### 3.7 Molecular experiments to Identify *A. fumigatus* and *C.albicans* using specific gene:

Using the universal primers species specific primer to identify the most common infectious fungi causing LRT infection to the species level the PCR was performed:

### ***Afumi* gene**

The PCR reaction was targeted against synthesis of gene (*Afumi*) as the results demonstrated that *Afumi* primer molecular weight 400bp had a high sensitivity to detect all pathogenic *A. fumigatus* isolated (1).



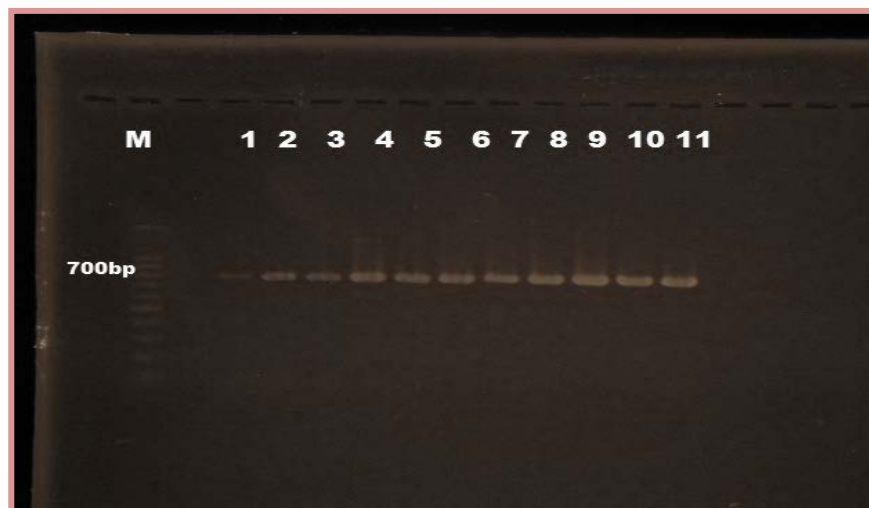
**(Fig. 1):** Agarose gel electrophoresis of PCR amplification products of *Afumi* gene in *Aspergillus fumigatus* (1.5% agarose, 100V, 90 min.) M: 100 bp DNA ladder. Lane 1-11 *Aspergillus fumigatus* isolated throughout the study.

A. *fumigatus* isolates used as template and PCR carried out with the primer for portion of the target gene of *A. fumigatus*.

The present result is in concomitant with many other studies in relation to the diagnosis of *A. fumigatus* based on virulence factors that qualify it to be the most prevalent. The adhesion factor is the most important factor which play a major role in the colonization of *A. fumigatus* in different tissues in human body specially respiratory system which the first target of allergic fungi. The prevalence of *Afumi* gene has been confirmed amongst *A. fumigatus* isolates. It also has demonstrated that adhesion is an important and relevant virulence factor and that it can also contribute to virulence in *A. fumigatus* [54].

### ***CABF59* gene**

For each isolated culture identified by PCR a specific primer pairs of *C. albicans* was used as the genomic sequences of DNA topoisomerase-II gene. Conventional PCR used for identification of virulence genes (*CABF59*) in *C. albicans* *CABF59* gene appeared in all of isolates, with molecular weight 700 pb as in Figures (2).



**(Fig. 2):** Agarose gel electrophoresis of PCR amplification products of CABF59 gene in *C. albicans* (1.5% agarose, 100V, 90 min.) M: 100 bp DNA ladder. Lane 1-11 *C. albicans* isolated throughout the study.

In this study, a rapid, simple and reliable PCR system for the definitive identification of pathogenic *C. albicans* has been used. The positive result obtained in using PCR method as the universal fungal primer in amplifying the *C. albicans* specific primer has confirmed the above results. Therefore, the results in figure (4.7-B) revealed that all the isolates had given a band with a molecular weight (700bp) confirming their relevance to *C. albicans*. Thus the PCR amplification method to identify those fungi that cause LRT infection has been approved.

The high percentage of positive results obtained by genetic method might be contributed to the possibility that patients on antibiotic treatment make the process of identification even difficult. This is in concomitant with [55] who reported that 56% their patients referred to their center were already on topical antibiotic therapy before culture specimens were collected.

These results were close to those of [56] who found that the positivity of PCR assays in the LRT infections cases had reached 70%. It has also been reported that the standard techniques for culture in fungal infections are complicated due to some factors i.e. slow growing of fungi, unfreshly prepared media, high chance of growth contaminants and the time involved in confirming the culture growth. The culture is positive only if the specimens contain viable organism while a PCR based test detect both viable and non-viable organisms.

[57] reported that PCR was more sensitive as a diagnostic aid for fungal infection, when fungal DNA was detected in 51% out of 65 specimens tested, whereas the culture method was positive in 43.1%. Thus, the universal fungal primer used was suitable for amplification of fungus specific DNA fragments in the sputum of patients. Based on the present results it might therefore, be concluded that PCR can be recommended as a rapid and sensitive diagnostic technique for LRT infection and allergic infection and the fungal environmental pollution like in door fungi.

For fungi the time taken by PCR assay was 4-8 hrs. whereas positive fungal cultures took 1-8 days. Identification at species level by the molecular methods was possible in all cases. The PCR not only proved to

be an effective rapid method for the diagnosis of fungi caused LRT infection, but was also more sensitive than the stain and culture methods. It has also been mentioned that the fungal PCR must be added as the screening diagnosis test when an early infection suspected, and the molecular identification as the gold standard technique for the identification of cheats' fungal pathogens.

#### 4. Conclusion

It is concluded that:

1. Fungal species isolates from patients do appear similar to tuberculosis TB, as causative factor for LRTI.
2. The sources of fungal isolates originated from both out and indoor.
3. Direct examination and culturing of sputum are important technique to diagnosis the allergic fungi.
4. The isolates of both *Aspergillus fumigatus* and *Candida albicans* were diagnosed upon similar bands at molecular levels and that PCR is very important to confirm diagnosis of allergic fungi.

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